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HYPERACTIVATION OF THE NEUROHYPOPHYSIS AS THE PATHOLOGICAL BASIS OF ECLAMPSIA AND OTHER HYPERTENSIVE STATES *

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An excessive infiltration of the neurohypophysis by epithelial elements, bearing a certain resemblance to the cellular invasion of a malignant tumor, appears to have been first mentioned by Thom in 1901,[45] since when the condition has been observed and commented on from one aspect or another by many others. There has, however, been a difference of opinion regarding the precise nature as well as source of origin of the inwandering cells, and still less agreement as to the meaning of the process. Indeed, it has not been generally assumed to have any physiological or pathological significance.

In the lower animals a patent cleft, the relic of Rathke's pouch, divides the epithelial portion of the pituitary body into a bulky pars distalis and a thin pars intermedia, which serves closely to envelop the pars nervosa proper. The posterior lobe or neurohypophysis is thus composed of two easily recognizable but mechanically inseparable portions.†

* These studies, made in the Surgical Laboratory of the Peter Bent Brigham Hospital with the assistance of Dr. Louise Eisenhardt, were the basis of the first lecture before the Medical Research Society delivered at University College, London, November 2, 1933.

† Closely embracing the pituitary stalk and lower tuber, both in animals and in man, is a tongue-like prolongation of the epithelial lobe known as the pars tuberalis, of whose independent secretory function even less is known than of the pars intermedia. The chief difficulty encountered by those who have attempted, by study of its extracts, to determine the separate function of the pars tuberalis has possibly lain in the fact that the large venous trunks which pass through it contain variable amounts of the secretory product both of pars distalis and of pars intermedia in the process of transport to the tuberal nuclei.

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In the higher anthropoids and in man, on the other hand, because of the practical disappearance of the cleft there exists no such clear anatomical distinction between pars distalis and pars intermedia. Consequently, many writers (*e. g.*, Plaut[36] in 1922, Erdheim[17] in 1925, Dayton[14] in 1926, Benda[5] in 1927, and Kraus[27] in 1928) have expressed the belief that the latter has become so rudimentary or vestigial it is futile to consider the two epithelial parts of the human gland other than as a whole. This has been particularly emphasized during the past several years by Berblinger,[6, 7] who distinguishes glandular hypophysis from neural hypophysis but disclaims any recognizable subdivisions of the former. Hence, in accordance with this view, any cells that wander into the pars nervosa must come from the pars distalis or anterior lobe proper.

Several of the authors who adhere to this opinion, more especially Kraus and Berblinger, have examined large numbers of human glands removed at autopsy and have made detailed estimates of the relative percentage of basophilic elements present in the pituitary body as a whole under various conditions of disease. Berblinger's computations of an increase or diminution of these elements are based on their relative number, irrespective of the lobe in which they occur. Kraus,[27] on the other hand, lists those of anterior and posterior lobe separately and it would appear from his tables that the pars nervosa often harbors basophilic elements, particularly in association with what he calls the hypersthenic constitution.[28]

However, from my reading of their papers it does not appear that either of these distinguished writers on the subject looks upon the basophilic infiltration of the pars nervosa as anything more than a fortuitous overflow of these elements from the pars anterior. Berblinger, indeed, emphatically insists that pituitary disease is entirely an anterior lobe problem. Just why the acidophilic elements of this lobe, which in man are heavily massed in the region adjacent to the site of the original cleft, fail similarly to infiltrate the nervous tissue is not explained.

A contrary view, with which the writer sides, is held by another group (*e. g.*, Tölken[46] in 1912, Schönig[40] in 1926, Lewis and Lee[30] in 1927, Marburg[31] in 1929, Biedl[8] in 1929, Aschoff[3] in 1930, Rasmussen[38] in 1930, Orlandi[34] in 1930, Guizzetti[20] in 1933). While granting its relatively inconspicuous nature in man, most of these authors nevertheless maintain that the pars intermedia is anatomi-

cally recognizable by the distinctive character of its cells, which acquire basophilic granules in the process of maturation and migrate into the pars nervosa. Though the basophils arising from this source possibly tend to be somewhat smaller, to have a more pyknotic type of nucleus and a less heavily granular and less abundantly vacuolated cytoplasm than the basophilic elements of the pars distalis, in the terminal stages of ripening the basophils from either source are morphologically indistinguishable.

These differing points of view regarding the source of origin of the basophilic elements occasionally observed in the pars nervosa would seem to be of less importance than the physiological significance of the process; and it does not appear to have been suggested, or at least not to have been emphasized, by either party in this contention that *the degree of basophilic infiltration may represent a measure of neurohypophysial activation*. It is proposed herein to lay stress on this fundamentally important point.

That the posterior lobe contains an active principle, not found in the anterior lobe, has been known since Howell's discovery in 1898 of a pressor substance in its extracts. However, all posterior lobe extracts obtained from the lower animals are necessarily products of both pars intermedia and pars nervosa. And since it is inconceivable that the neural tissue composing the tubero-infundibular apparatus is capable independently of elaborating a hormone, the active principle in extracts of the lobe must obviously be derived from its cellular investment. It certainly could not come from the pars distalis, for in the lower animals from which these extracts are customarily made the two lobes of the gland are readily separated.

Herring [21] was the first to study and describe the peculiar manner of posterior lobe secretion. In the gland of the cat he observed the casting off from the pars intermedia of cells which in their passage through the pars nervosa become transformed into hyaline-like masses. These masses of secretory product, in favourable preparations, not only are discernible in the posterior lobe of normal glands but may be increased visibly under certain experimental conditions such, for example, as after a preceding thyroidectomy.[22] While Herring's conception of the process has been looked upon with skepticism in many quarters and may need some slight reinterpretation, a number of authors (*e. g.*, Sharpey-Schafer,[41] Cushing and Goetsch,[13] da Costa,[10] and Remy Collin [9]) have agreed with him in

all essential points. His views unquestionably furnish the only satisfactory explanation of the normal manner of neurohypophysial activity.

As mentioned in the introductory paragraph, a massive infiltration by basophilic elements is not infrequently seen in the posterior lobe of man. However, not even by those who believe in a persistent pars intermedia and look upon the invading elements as derived from it does the suggestion appear to have been made that the degree of infiltration is a measure of functional activity and that an excess of posterior lobe (*i. e.* pars intermedia) secretion should be recoverable from glands in which the process is marked.

It has been pointed out separately by Erdheim, Tölken, Kraus and Berblinger that an increase of basophilic elements in the gland as a whole is an accompaniment of advancing years of life when naturally enough it is often associated with atherosclerosis and renal disease. They appear, however, to regard the process as merely coincidental with these disorders and do not look upon it as in any sense an aetiological factor in their production, unless such a statement has been overlooked. In his recent monograph Berblinger [7] emphatically states (page 936): "Neither Hoeppli nor I ever claimed that the increase in the basophilic cells represents a pathological finding in the hypophysis but on the contrary, we regarded the cellular variation as a reaction that supposedly bears some sort of relationship to the altered renal activity."

Of the several pathologists who have dealt with the subject Skubiszewski [42] (1925) appears to have been the only one to have grasped the idea that posterior lobe basophilia might be an indication of hyperfunction. He expressed the belief that the cardiac hypertrophy and diuresis accompanying chronic interstitial nephritis might thus be accounted for. This view appears to have been based on the assumption that the posterior lobe principle had a diuretic rather than an antidiuretic action. But however this may be, the time was not ripe for such an interpretation and Berblinger promptly opposed it on the ground that in uraemia with lowered diuresis the same picture is particularly frequent. As a matter of fact, only in the past few years through the discovery of a posterior lobe-like substance in the blood in certain hypertensive states could the full significance of hyperactivation of the posterior lobe by the invading elements have been thoroughly grasped.

1. Neurohypophysial Activation in Pituitary Basophilism

What has led to a revival of interest in this particular matter has been the postmortem disclosure, in a case of what has been termed pituitary basophilism,[11] not only of a definite basophilic adenoma in the pars distalis, but of an excessive invasion of the pars nervosa by elements of the same type (Fig. 1). This strongly suggested a dual source of the symptom-complex as partly anterior hypophysial and partly posterior hypophysial. For in these clinical states not only is there evidence of gonadal dysfunction but the adiposity, glycosuria, pigmentation of the skin, hypertension and ultimate atherosclerosis might well be ascribed to hyperfunction of the posterior lobe.

This disclosure naturally led to the re-examination of sections of the pituitary glands from the known victims of the disorder obtained from various sources; and though in the single sections from the Anderson case, the Parkes Weber case, and one or two others, no notable basophilic invasion of the posterior lobe is seen, it is very marked in the gland from the Raab-Kraus case, of which Professor Kraus has kindly submitted four sections. Indeed, on further study of this case, the interpretation of which has been thoroughly discussed in the literature both by Kraus [26] and by Raab,[37] it is my impression that the tumor is an actual adenoma of the pars intermedia (Fig. 2).

While this interpretation of the lesion is not in accord with that held by Dr. Kraus, to whose opinion and wide experience I should naturally wish to defer, certain reasons in its favour may be given. Not only is a rich basophilic invasion of the pars nervosa taking place from the periphery of the adenoma, as he has clearly pointed out, but there is also a tendency in this direction in areas remote from the tumor where the investing pars intermedia is separated from the pars anterior by a large Rathke's cyst (Fig. 3). It would seem, therefore, that the cells must come from the pars intermedia rather than the pars distalis from which, indeed, the adenoma itself is sharply delimited. A further reason lies in the fact that an adenoma of similar sort, unmistakably from pars intermedia, has been observed in a fatal case of eclampsia to be described (p. 156).

The vasculo-renal changes incidental to old age have, as already stated, been shown to be accompanied by an increase of basophilic elements of the pituitary body as a whole. But since hypertension

and atherosclerosis of pituitary basophilism occur in young persons, it was natural to assume that the basophilic adenoma was the causal agent rather than a resultant effect. What is more, since extracts of the posterior lobe alone contain a demonstrable pressor substance, the conclusion was inescapable that the posterior lobe basophilia was the important factor in the hypertension, rather than the numerical increase of these cells in the pars distalis.

2. Neurohypophysial Activation in Eclampsia

We may now turn to evidence from another source. Several years ago (1918) it was pointed out by Hofbauer [23] that the diminished output of urine, the oedemas, convulsions and vascular hypertension characterizing eclampsia strongly suggested an intoxication by excess of posterior lobe secretion. In pursuit of this hypothesis Anselmino and his collaborators [1, 2] have found an antidiuretic substance in the blood of patients with eclampsia, and also a pressor substance in all instances when systolic blood pressures of 180 or over were a feature of the syndrome.

In view of these interesting observations it was anticipated that the same excessive infiltration of the pars nervosa, which was so striking in the case of pituitary basophilism with hypertension and nephrosclerosis, might also be present in the pituitary glands of patients with eclampsia.

A hint that this idea might be worth pursuing was afforded by the sections of a gland in which such an infiltration had been observed (Fig. 4). The specimen came from the Boston Lying-In Hospital and the sections have been filed in our laboratory since 1921, when Percival Bailey [4] was in search of a tinctorial method of distinguishing the granules in basophilic and acidophilic cells. There unfortunately is no history of the specimen and it is not definitely known to have come from an eclamptic patient, but considering its source the probabilities are that it did.

Since Erdheim and Stumme's classical paper [18] (1909), in which the pregnancy cells were first described, most pathologists who have studied the pituitary body in pregnancy have been more interested in the condition of the glandular than of the neural hypophysis. Though Erdheim had been among the first to describe the basophilic invasion of the posterior lobe no reference is made to its occurrence in any of the eighteen cases of eclampsia that he and Stumme in-

cluded in their important monograph. On the other hand, in three of the twenty-five glands in their control series (two cases of nephritis and one of biliary carcinoma) such an invasion is specifically mentioned. The professor of pathology in another university has kindly forwarded for study single sections of the pituitary bodies from eleven cases of eclampsia in his collection. Five of them fail to show the posterior lobe at all, and in none of those that happen to retain portions of it does any active cellular invasion appear. All this would seem to constitute overwhelming evidence against the view that posterior lobe activation by basophils might be a factor in the toxaemias of pregnancy.

Not only are pituitary glands of eclampsia difficult to come by, but it would appear that the "toxicoses of pregnancy" and "eclampsia" are exceedingly vague terms in obstetrical parlance. During the past few months we have succeeded in securing nine uncut glands supposedly from fatal cases of the disorder. While not all of them show basophilia of the posterior lobe, some of them do, and in a few instances it is excessive. To a consideration of these cases we may now turn.

For the first two specimens which came from the pathological department of the Boston Lying-In Hospital I am indebted to my colleague, Professor S. B. Wolbach.

CASE 1. The patient, 28 years of age, had been under treatment for advanced diabetes mellitus for a period of 6 years. She was sent to the hospital a few hours after a normal parturition because of a sudden convulsion with subsequent coma. The systolic blood pressure was 170; the urine showed large traces of albumin and the blood a marked hyperglycaemia. She was twice subjected to plasmapheresis with fatal issue. While the diagnosis was "puerperal toxaemia with convulsions," there was some question as to whether the death might not have been due to diabetic coma.

The serial sections of the *pituitary body* show no posterior lobe invasion whatsoever.

The history of the second case from the same source is as follows.

CASE 2. On March 29, 1931, an 8 months pregnant primipara, aged 34 years, was brought to the hospital in a comatose condition after having had three consecutive convulsions. The urine drawn by catheter was small in amount and showed a large trace of albumin. Plasmapheresis was performed and she was given hypertonic glucose and saline solution. The blood pressure was variable, the highest reading having been 190/100. The lowest reading of 50/38 was taken shortly before death, which occurred on the day after admission, the patient never having regained consciousness.

The postmortem examination showed: an 8 months undelivered fetus, acute tubular nephritis, slight fatty infiltration of the liver with central congestion and necrosis, acute toxic splenitis, pulmonary congestion and oedema with (?) terminal bronchopneumonia, generalized slight arteriosclerosis, and follicular desquamation of the thyroid.

On its removal in 1931 the *pituitary body* had been cut sagittally in halves, one of which was preserved in alcohol, the other in Zenker's fluid. On being sectioned serially the *anterior lobe* shows a great abundance of basophils, often in large clusters, some of almost adenomatous-like character. There is no necrosis, scarring or round-cell infiltration.

In the *posterior lobe* (evidently cut into and partly lost in process of removal) the distinction between pars distalis and pars intermedia is easily drawn by long, narrow, colloid-containing cavities representing the original cleft. In the more lateral regions the cleft may be followed all the way through to its open mouth in the dura. Because of this separation it is difficult to imagine that the fairly abundant basophilic infiltration of the posterior lobe (Fig. 5) represents an overflow from the pars distalis.

The colloid-filled cleft extends from the base of the gland four-fifths of the way up toward the stalk. Above this level the distinction between pars distalis and pars intermedia is less clear. Below this level signs of reactive hyperplasia of the pars intermedia are everywhere evident; the acini have increased in number and ripened basophilic elements are being cast off to invade the adjacent nervous tissue. Fortunately the hyaline masses have not been wholly dissolved out of the tissue and the pars nervosa is everywhere heavily charged with them, large accumulations being present in certain regions (Fig. 6).

For the next three specimens to be described thanks are due to Dr. G. Elliott May of the Boston City Hospital.

CASE 3. On Jan. 30, 1933, Mrs. A. F., a primipara 42 years of age, first consulted Dr. May when about 7 months pregnant. For 2 months she had been having frequent vomiting attacks, which were ascribed to indigestion from which she had suffered for years. Latterly she had voided infrequently and for the past week only very small amounts.

She was emaciated and dehydrated, having a dry, coated tongue, slight oedema of the ankles and a blood pressure of 160/100. The urine showed a trace

of albumin, a few red cells and occasional granular casts. Test of renal function showed it to be low; the non-protein nitrogen was 36 mg. per cent. She was immediately sent to the hospital with the diagnosis of toxaemia of pregnancy and hyperemesis gravidarum.

During the next week under forced fluids she improved greatly. The urine increased in amount, the oedema disappeared and the vomiting ceased. Her blood pressure, however, progressively rose to 184/128 by February 7th, on which day she began having labour pains. Of these she complained so bitterly she was given phenobarbital and scopolamine in small doses. Later in the day she passed into a coma from which she never aroused. On the afternoon of February 8th she was delivered normally, with the aid of an injection of pituitrin, of a still-born foetus. The systolic blood pressure dropped from 182 to 138 and 2 hours later to 90/70. She remained comatose, in spite of all efforts to relieve the condition, until her death on the afternoon of Febuary 10th.

The postmortem examination disclosed an apopleptic clot in the right frontal lobe of the cerebrum, a duodenal ulcer, and multiple small abscesses of liver and kidneys. There was no atherosclerosis. The diagnosis was "non-convulsive eclampsia without typical autopsy findings."

The *pituitary body* had been cut in two in a sagittal plane, the stalk having been destroyed in the process; one-half had been fixed in Zenker's fluid, the other in formalin. Serial sections of each block were made in the vertical plane of the original cut.

The *pars distalis* shows near its anterior edge an irregularly marginal area of necrosis about 3 mm. in diameter. Infiltration with polymorphonuclear leukocytes has begun to take place in the necrotic area, which is encircled by alveolated clusters of basophilic cells more sharply outlined than usual because of their separation by oedematous strands of interstitial tissue.

Throughout the pars distalis basophils far outnumber the acidophilic elements, the latter being largely confined to a broad juxtaneural crescentic strip. Many large chromophobe elements (gestation cells?) are scattered through the lobe and one gains the impression that they are ripening into pale staining basophils.

The *pars intermedia* is clearly separated from the pars distalis, throughout most of the sections, by the overabundance of colloid in the cleft. There is an extensive posterior lobe invasion (Fig. 7) by basophilic elements from the pars intermedia, more particularly from the lower third of the cleft. The column of cells extends in the usual conical fashion halfway through the lobe.

Throughout the *pars nervosa* the hyaline masses happen to have been unusually well retained in the interstices of the neural tissue

(Fig. 8), many of them adjacent to the tongues of the still living cells showing ghosts of nuclei. The hyaline masses can be followed easily as they stream toward the direction of the stalk. The abundant hyalin (colloid) in the cleft appears to come from the same cellular elements. It can be seen emerging from the mouth of the cleft into the subarachnoid spaces.

In all three of the foregoing cases the gland, before it was received, had been divided on a sagittal plane, the two halves having been placed in different fixatives. This procedure, for reasons given elsewhere,[2] adds difficulties of interpretation to the study of the serial sections from the loss of topographical relations. Wishing to obtain an entire gland with its hypothalamic attachment intact, Dr. May kindly notified me of the autopsy on the following case and I was permitted to remove and preserve the block of tissue in the desired way.

CASE 4. The patient, an exceedingly adipose multipara 38 years of age, was admitted to the Boston City Hospital June 6, 1933. Three years before she had been attended by her local physician in her ninth pregnancy. At that time she had a normal parturition, though she was found to have a blood pressure of 180/100. As this condition subsequently persisted, it was looked upon as an essential hypertension.

During this, her tenth pregnancy, the systolic pressures had varied from 200 to 220. She latterly had been having much nausea and vomiting with swelling of the hands and feet.

Before her admission she had been in labour for several hours with a breech presentation and, becoming hysterical, she was finally taken to the hospital. There she was found to have a blood pressure of 210/120, going up to 260/120 during her pains. The delivery of the child was tardy and subsequently the mother passed into a comatose state without convulsions and died in a few hours. The clinical diagnosis was "toxaemia of pregnancy."

The autopsy showed very little apart from a moderate cardiac hypertrophy and dilatation, slight atherosclerosis, fatty infiltration of the liver and acute pulmonary congestion.

The *pituitary body*, a large, succulent gland (not separately weighed) with its stalk, tuber and block of the hypothalamic region, was removed in one piece (Fig. 9), fixed in formalin, serially sectioned in the horizontal plane and stained with haematoxylin and eosin.

The posterior lobe in its lower portion shows a cellular invasion by basophilic elements that almost surround its circumference (Fig. 10). A large excess of colloid in certain parts of the cleft has broken widely

into the pars distalis. In many areas the infiltration is massive (Fig. 11) and strands of normally staining basophils can be followed well up into the stalk (Fig. 12). The holocrine secretion has been well preserved in between the infiltrating tongues of viable cells (Fig. 13). The tuber is broken up into widely opened spaces as the tip of the infundibular cavity is approached. This, as usual, shows a highly defective ependymal cuticle.

The story of Dr. May's third case is briefly as follows.

CASE 5. On Aug. 12, 1933, a 23 year old Polish woman, about 7 months pregnant, who had had no prenatal care, was admitted to the Boston City Hospital with the story that she had recently shown some swelling of the face and ankles, and for 24 hours had been having a series of convulsive seizures. She was unconscious on admission and about 3 ounces of urine were obtained by catheter, showing a heavy trace of albumin, hyaline and granular casts and red cells. Her blood pressure was 170/110. She remained in deep coma in spite of treatment and died 36 hours later.

The postmortem examination showed lesions in the liver and kidneys typical of eclampsia. There was also an intense venous congestion of the cortical vessels of the brain with a small subarachnoid hemorrhage over the right occipito-parietal region.

The *pituitary body* with the hypothalamus had been removed in a single block, fixed in formalin and forwarded for study. The gland was large; both stalk and tuber were swollen and succulent. After serial sectioning in the horizontal plane not only are basophiles found to be abundant in the pars distalis, but from the pars intermedia two cones of these same elements project into the pars nervosa (Fig. 14), from one of which ripened cells can be traced well into the center of the lobe. The pars intermedia in other regions shows an abundance of Rathke's cysts lined by ripened basophiles (Fig. 15) which have broken into the cysts as well as the cleft and are scantily invading the pars nervosa.

In the Kraus-Raab case of pituitary basophilism, as previously stated, there was found what I have ventured to interpret as a basophilic adenoma of the pars intermedia (*cf.* Fig. 2). Some hesitation was felt in regard to this for the reason that no such adenoma of this epithelial zone had been definitely described. However, the disclosure of a similar lesion in the gland of a patient with eclampsia makes the given interpretation of the case seem the more probable.

Through my one-time pupil, Dr. Benno Schlesinger, some inquiries were made regarding the prevalence of eclampsia in Vienna. Desiring to interest Professor Erdheim in the subject at hand and with the hope that his old eclampsia sections might be gone over to see what proportion of them showed invasion of the type in question, a photomicrograph of one of our sections was sent in illustration of what was to be looked for. He replied that he had never seen any corresponding degree of "spreading out" of basophilic cells in the posterior lobe, except in the glands of old persons. Unfortunately his old slides had been thrown away and for years he had had no opportunity further to pursue his studies of pregnancy.

Dr. Schlesinger made further inquiries and learned at the Allgemeines Krankenhaus that they do not have more than one or two fatal cases of eclampsia each year. He subsequently, from another source, had the good fortune to secure and forward to me the gland to be described.

CASE 6. The patient, a primipara aged 37 years, was admitted to the Brigittaspital of Vienna May 2, 1933. She had marked hypertension, the systolic registrations ranging between 210 to 180 during the next 10 days. On May 13th she had six convulsive seizures and at 6 P.M. the child was delivered by forceps extraction. In spite of stimulants she failed to rally and died 8 hours later.

The postmortem examination disclosed a "gray and fragile" liver, a parenchymatous and fatty degeneration of the kidneys, excentric hypertrophy of the left ventricle, acute oedema of the lungs, and oedema of the leptomeninges.

The *pituitary body* was large, ovoid, and weighed *circa* 960 mg. The prominent posterior lobe had been slightly damaged in removal; there was an obvious extrusion of a large hyaline globule in the cleft between the posterior and anterior lobes.

Serial sections on a horizontal plane were cut at 8 microns, every tenth section being mounted and stained with haematoxylin and eosin. The first thing noticeable is the large, full pars distalis which shows no areas of necrosis or round-cell infiltration. Basophilic elements abound, many of them in large clusters. The transverse cleft is distended with colloid which has burst through into the meninges. It cleanly separates pars distalis from pars intermedia (Fig. 16). The posterior lobe at this level is defective but an extensive invasion from pars intermedia is clearly apparent (Fig. 17).

The cells from this low-level invasion pass upward and backward toward the posterior portion of the pars nervosa where they become

merged with a large, sharply defined cellular mass (Fig. 18). This globular lesion is readily visible to the naked eye from the 8 micron sections No. 720 to 2250 (Fig. 19), its maximal diameters being about 3 by 4 mm. It proves on higher magnification to have the architectural features of an adenoma (Fig. 20) and its component elements are unmistakably fully ripened basophilic cells (Fig. 21).

The glands of the two following cases were received through the courtesy of Dr. C. B. Courville of Los Angeles.

CASE 7. The patient, an obese woman aged 35 years, and 7 months pregnant, was admitted to the Los Angeles General Hospital May 13, 1933, having had three convulsions the previous day. She had had no prenatal care. There was some oedema of the ankles, feet and face. The urine showed a large trace of albumin and finely granular casts. The blood pressure was only 130/90. On May 18th she was delivered of a premature child. On May 19th she became comatose with Cheyne-Stokes respiration and was found to have a bilateral papilloedema. On May 22nd she died. The case was looked upon as one of typical postpartum eclampsia.

At autopsy changes in the liver and kidneys were found consistent with eclampsia and there were in addition multiple focal haemorrhages in the brain.

The *pituitary body* shows very little change. Posterior lobe invasion is slight, occurs in one small area only (Fig. 22) and there is no colloid in the cleft.

In the following, the second of Dr. Courville's cases, there was doubt of the diagnosis.

CASE 8. The patient, a multipara 7 months pregnant, was admitted to the Los Angeles General Hospital on June 15, 1933, in status epilepticus from rapidly recurring right-sided fits. She had been known to have convulsions previously of Jacksonian type beginning on the right side. The blood pressure was 165/100. The urine showed a trace of albumin and a few casts. The cerebrospinal fluid was blood-tinged and under tension. A diagnosis of subdural haemorrhage was made and an operation performed without disclosing a clot.

The autopsy showed cerebral oedema with petechial haemorrhages, a thickened arachnoid and an apparent thrombosis of the left middle cerebral artery. The case was looked upon as "atypical eclampsia."

The *pituitary body* on section shows (Fig. 23) only a very slight degree of posterior lobe invasion in one place. There is certainly no excessive basophilia in either anterior or posterior lobe.

The records and specimen from the last of the cases have been kindly forwarded by Dr. Frank Forry of the Indiana University Medical School.

CASE 9. The patient, 44 years of age, was an obese multipara in the 8th month of her ninth pregnancy. She had been known to have hypertension for several years. She was admitted to the hospital Aug. 16, 1931, in deep coma with cyanosis. There was oedema of the extremities. The urine showed a large trace of sugar, albumin, red cells and granular casts. The blood pressure, taken frequently, ranged from 238/130 to 190/110. She was spontaneously delivered of a still-born child and died 3 days after admission in a state of hyperthermia (107° F).

At autopsy focal necroses of the liver, chronic nephritis and bronchopneumonia were found. There was no question of the diagnosis of eclampsia gravidarum.

The *pituitary body* shows a massive posterior lobe invasion, as heavy as that shown in Figure 5. The gland unfortunately was fragmented in removal and the sections stain so feebly the photomicrograph is not worth reproducing.

Summary

Briefly summarized, six of these nine cases (Nos. 2, 4, 5, 6, 7 and 9) were typical of eclampsia and in the four (Nos. 2, 4, 6 and 9) that had shown marked hypertension an excessive basophilic invasion was present; Case 5 showed only a moderate invasion with the systolic pressure not above 170, and in Case 7 there was no hypertension and very slight invasion.

In the other three cases (Nos. 1, 3 and 8) the diagnosis of eclampsia was questionable or the condition atypical. There was no invasion in Case 1 with a systolic pressure of 170, and in Case 8 with a pressure of 165 it was slight. In Case 3, on the other hand, with a pressure of 184, the posterior lobe basophilia was marked. In all the cases, therefore, in which blood pressure registrations were 180 or over, there was marked basophilic infiltration of the posterior lobe. It will be recalled that Anselmino and Hoffmann found a pressor substance in the blood of eclamptics only when systolic pressures exceeding 180 were recorded.

3. Essential Hypertension in the Prime of Life

If I am correctly informed, it is generally recognized by obstetricians that when the toxaemias of pregnancy are accompanied by hypertension their victims are apt to retain an abnormally high blood pressure which is likely to be increased in each subsequent period of child-bearing. Alongside of this goes a tendency toward adiposity, examples of such a sequence being given by Cases 4 and 9 in the preceding series. So-called essential hypertension, however, is a common disorder by no means limited to such a small group, for it may victimize women who have never borne children and no less frequently men in the prime of life.

The postmortem examination on such cases often fails to show any satisfactory explanation for the patient's death. The usual finding on which the pathological diagnosis is based is a chronic progressive renal lesion characterized by hyalinoid thickening of the terminal arterioles. While these vascular changes may be more pronounced in the kidney than elsewhere and may possibly first be detected there, the process nevertheless is universal and similar arteriolar changes are found in all other organs. This was clearly pointed out sixty years ago by Gull and Sutton, whose important studies were the starting point of the vast amount of work that has since been done on arteriosclerosis and hypertension. Nevertheless, many clinicians are still inclined to regard essential hypertension (the "hyperpiesia" of Clifford Allbutt) as primarily a nephrovascular disorder, in view of the presence of albumin and casts in the urine.

While Gull and Sutton admitted complete ignorance as to the cause of their "arterio-capillary fibrosis" other than that it was common in old age and premature senility, it would have interested them to know that posterior lobe extract exerts its constricting effects on the peripheral arterioles and capillaries where the pathological changes they described primarily appear. What is more, as pointed out by Professor Harold E. MacMahon, precisely the same renal lesion, variously called progressive vascular nephritis and malignant nephrosclerosis, may be seen to follow both hyperpiesia and pituitary basophilism, and it is quite possible that the more acute renal lesions of eclampsia are of the same order.

In view of what has gone before, it was natural enough to suspect that posterior lobe basophilia might also accompany these conditions

of so-called essential hypertension. The first opportunity to examine the pituitary body from such a case in the desired way was afforded by Dr. George Hass, the resident pathologist of the Peter Bent Brigham Hospital, who removed the gland and anterior hypothalamus in one piece from the body of the man whose story follows.

CASE 10. Edward M., aged 45 years, a negro chef of good family history and exemplary habits, entered the medical wards of the hospital Feb. 8, 1933, complaining of precordial pain, shortness of breath, inappetence, and recent loss of weight. For *2 years* he had been having morning headaches regressing during the day; for *3 months* dyspnoea on exertion, often associated with substernal pain radiating to the left shoulder and ceasing abruptly; also attacks of nocturnal dyspnoea with productive cough; for *2 months* transient attacks of blindness in the right eye, lasting a few hours; for *2 weeks* occasional slight epistaxis.

The physical examination revealed a cardiac enlargement and an expanded aorta (shown by the X-ray), with soft systolic murmur and accentuated second sound. The blood pressure was high, varying around 230/160. The urine showed the slightest possible trace of albumin, an occasional red blood corpuscle, rarely a hyaline cast. The Wassermann reaction was positive for the blood (repeated), negative for the spinal fluid. There was no history of a syphilitic infection.

He was abundantly studied by many observers during the following 6 weeks with the diagnosis of syphilitic aortitis chiefly favoured, though some thought it was coronary disease, others a nephrovascular disorder. He had occasional attacks of severe pain, substernal or epigastric, during which his blood pressure would usually fall, on one occasion to 135/80. For these attacks he was given nitroglycerine and often required morphia.

In the early morning of March 25th he was taken with a typical attack of agonizing epigastric pain, which sedatives failed to relieve. This continued during the day, with periodic vomiting and frequent watery bowel movements containing blood. His blood pressure gradually fell to low levels, he became dyspnoeic, and died 24 hours later.

The postmortem examination showed a moderate cardiac hypertrophy, a slight degree of atherosclerosis, a progressive vascular nephritis and acute haemorrhagic colitis. It otherwise was essentially negative. There was nothing to support the clinical diagnosis of luetic aortitis, coronary thrombosis or myocarditis, and no cause for the "anginal" attacks was apparent.

Grossly the *pituitary body* was small, concave above, and its two lobes easily distinguishable, the posterior lobe being unusually prominent. Serial sections were taken through the entire block, including the hypothalamus, from below upward.*

The *pars distalis* shows no discernible abnormalities. The clusters of basophils are as usual principally disposed toward the anterior

* The sections from this case were the basis of a recent paper on the secretory activity of the two lobes of the gland and manner of their discharge.[12]

surface of the lobe and are not pathologically numerous. The acidophils are chiefly massed in the deeper portions of the lobe.

The *pars intermedia*, despite the almost total absence of a cleft, is clearly distinguishable from the pars distalis by the well marked limiting zone of basophilic elements which almost everywhere, even up to the root of the stalk, are actively invading the pars nervosa, here and there sending heavy wedge-shaped columns of cells deeply into the lobe (Fig. 24).

The *arterioles* encountered in the sections of the hypothalamus show precisely the same changes as those affecting the vessels of the kidneys, so that the process is a general rather than a local one. Numerous minute capillary haemorrhages have occurred in the pars nervosa, stalk and tuber.

This case is one of several in which very similar conditions have been found. The patients have usually been of middle age, often obese, have shown marked vascular hypertension, enlargement of the heart, traces of albumin with a few casts, and rare renal elements in the urine. They have usually succumbed with symptoms of acute pulmonary oedema. The postmortem examination has shown malignant nephrosclerosis with cardiac hypertrophy and a more or less marked atherosclerosis. Fatty infiltration of the liver has been common, also macular or ulcerative lesions of the gastro-duodenal mucosa.

4. Hypertension with Atherosclerosis in the Aged

These are the conditions in which an increase of basophilic elements sometimes invading the posterior lobe have already been described by Kraus, Berblinger, Erdheim and others. Nowhere, however, does it appear to have been suggested that the cellular invasion of the neurohypophysis was an indication of posterior lobe activation that might be the primary factor in the hypertension, causing in its turn the progressive vascular and renal changes so frequent in aged persons.

The cases are so common specific examples need scarcely be given. Not only have several instances been met with in our own series but during the past few months, since local attention has been drawn to the matter, some of the younger pathologists in the several hospitals associated with the Harvard Medical School have begun routinely to study the *pituitary body* in all autopsies. Some of them have brought

specimens showing marked basophilia of the posterior lobe. Good examples of massive invasion occur in the following two cases submitted by Dr. John I. Bradley of the Massachusetts General Hospital.

CASE 11. The patient, a 60 year old labourer, had been known to have a high blood pressure for some time before his admission to the hospital on Sept. 21, 1932, following a cerebral accident. Though conscious and alert, his speech was thick and unintelligible. The blood pressure was 180/110 and the eyegrounds showed moderate tortuosity and sclerosis of the arteries. On the morning following his admission he suddenly became unconscious, the blood pressure fell to 60/50, and the body temperature rose to 108.2° F just before death.

The autopsy showed hypertrophy and dilatation of the left ventricle with marked generalized atherosclerosis. A thrombus was found occluding the basilar and right vertebral arteries, causing an infarct of the pons and multiple organized infarcts of the basal ganglia. There was also a pulmonary infarct with secondary oedema and congestion. The posterior hypophysis shows a marked basophilic invasion (Fig. 25) with distention by colloid of the adjacent Rathke's cysts.

CASE 12. A 67 year old multiparous Irish housewife was admitted to the hospital on April 1, 1933, because of intermittent vaginal bleeding for the preceding few weeks.

Examination showed an obese, arteriosclerotic woman with a blood pressure of 190/100. There was some swelling of the ankles and a slight trace of albumin in the urine without casts, and the phthalein excretion was 50 per cent in an hour. The cause of her complaint was found to be a large cervical polyp, and her hypertensive disorder was thought to be sufficiently well compensated to justify the risk of surgical intervention.

An operation accordingly was carried out on April 8th under gas oxygen and ether anaesthesia. Convalescence was uneventful and a few days later the patient was about to be discharged from the hospital when she suddenly collapsed, became unconscious, cyanotic and dyspnoeic. It was recognized that she probably had a pulmonary thrombosis and an emergency operation was carried out with the removal of a small embolus from the right branch of the pulmonary artery. This operation failed to accomplish its purpose.

The autopsy confirmed the clinical diagnosis of arteriosclerotic heart disease, endometrial polyp, and acute pulmonary embolism. In addition there was found a duodenal ulcer, a slightly enlarged heart, slight atheroma of the coronary arteries without constriction, and a moderate atheroma of the aorta.

The *pituitary body*, which was found to occupy a definitely enlarged sella, was small and flattened. It shows (Figs. 26 and 28) a massive basophilic invasion from the pars intermedia which is

visible to the naked eye. Everywhere between the viable cells the holocrine product is well preserved (Fig. 27), the shadows of the swollen nuclei being still discernible in many of the cast-off cytoplasmic masses.

Sections from a gland showing a degree of posterior lobe infiltration perhaps even more marked than in the preceding example have been kindly sent to me by Dr. John F. Noble, through the intermediation of Professor Rasmussen. The history of the case is as follows.

CASE 13. The patient, 90 years of age, was admitted to the Ancker Hospital of St. Paul on Jan. 13, 1933. Her past health had always been good and in her active years she had been the mother of twelve children. She was extremely obese and had a blood pressure of 176/110. She showed evidence of mental deterioration with marked excitation. Albumin was occasionally but not invariably present in the urine with a few hyaline casts. She died suddenly on March 16, 1933, supposedly from a coronary occlusion.

Postmortem examination showed excessive obesity, hypertrophy of the right heart, generalized atherosclerosis, and a terminal pulmonary thrombosis with marked oedema and congestion of the lungs.

The *pituitary body*, cut sagittally, proves to be a cup-shaped gland (Fig. 29) with a massive basophilic invasion occupying practically the entire anterior half of the pars nervosa (Fig. 30). The invading cells bud off in characteristic fashion from the vascular stalks (Figs. 31 and 32). These stalks show a larger amount of perivascular connective tissue than is usual and this may conceivably represent the consequences of a long-standing process with fluctuation in activity.

DISCUSSION

In venturing to interpret the posterior lobe basophilia of eclampsia, of essential hypertension, and of the atheroscleroses and nephropathies of the aged in terms of neurohypophysial activation, questions immediately arise which some attempt must be made to answer. How often does the process occur in persons of supposedly normal health? What, if any, is the relation of these basophilic elements of the pars nervosa to the cells of the pars distalis which appear to be identical in their tinctorial reactions and morphology? Are the invading basophils the source of all the recognized activities of extracts derived from the posterior lobe?

1. *The Frequency of the Process*: Doubtless some meaure of posterior lobe activity is constantly maintained. And if, as is assumed, the pars intermedia is responsible for it and the number of free basophils is an indication of its degree, few glands would, if serially cut, fail to show here and there an occasioned ripened cell wandering into the pars nervosa. But how often there occurs a massive invasion, as in some of the cases that have been cited, is impossible to say for want of routine postmortem studies of the gland with this particular point in view.

Only a few writers on the pathology of the hypophysis specifically mention these "inwandering" elements. Those who do, like Kiyono [25] (1926), merely allude to the fact without interpretation. Nor could there scarcely be any, for in his brief protocols of fifty-three cases thirty-two showed no invasion, twelve a slight invasion, and only nine a copious invasion. In this last group, four were examples of vascular disease, three of carcinoma of the breast, one had a brain tumor, and the ninth (the only subject below middle age) was a suicide. Rasmussen, in his valuable paper [38] (1930) dealing with the pars intermedia, depicts a single example of heavy infiltration without commenting on its possible significance. In a personal communication he states that in his collection of 240 serially cut glands a corresponding degree of invasion has been observed only half a dozen times.

The late Dr. Ernest Southard, for a number of years when pathologist to the Department of Mental Diseases, methodically collected and sectioned the pituitary bodies of the patients who had died in the Massachusetts state hospitals. His successor, Dr. Canavan, who continued to add to the material, has kindly permitted my coworker, Dr. Eisenhardt, to go through these sections to get a general idea of what they show. The glands were uniformly cut through the middle on the horizontal plane so the single sections of each that have been preserved are apt to transect the outer angles of the posterior lobe, where the cellular invasion in question is most likely to be seen.

Unfortunately the case histories that go with the specimens are brief. They chiefly relate to the mental status of the patients, and when factors such as blood pressure are mentioned it is difficult to tell when the reading may have been taken, for many of the patients had long been inmates of the institutions in which they died. But

leaving all else aside, in a series of 100 of these glands, 64 per cent showed no basophilic invasion of the pars nervosa whatsoever, 23 per cent showed a slight invasion, and 13 per cent showed a marked invasion. The average age of the thirteen cases was 56 years, the ages ranging from 34 to 83. In only two instances was the age below 40: one was a man of 34 who died of lobar pneumonia, the other a woman of 38 with a blood pressure of 170/90 and a postencephalitic syndrome. The conditions in the other cases were so variable as to baffle analysis. Naturally many of the older patients were found at autopsy to have had arteriovascular disease.*

As a check on this series of Dr. Southard's, sections from a large collection of pituitary bodies made at the Johns Hopkins Hospital many years ago have been gone over, those in which the posterior lobe does not happen to be well shown having been excluded. A consecutive series of 100 of these sections from different glands shows in sixty-two no basophilic infiltration, in twenty-two a few invading elements, in nine a moderately well marked invasion, and in only seven a heavy invasion. Serial sections would of course have increased the number of positive cases. As matters stand the percentages in this and in the Southard series are surprisingly close.

Much depends naturally on what the terms "slight," "moderate" and "heavy" indicate, and without suitable illustrations different writers might have different views on the matter. However this may be, it may be gathered that in the general run of autopsies a heavy posterior lobe invasion is not infrequent. For though Rasmussen's estimate is low, namely, 2.5 per cent, my series showed 7 per cent and the Southard series 13 per cent, while in Kiyono's smaller group of cases 17 per cent showed marked invasion.

2. *The Function of the Pars Intermedia*: In accordance with the view that the pars intermedia must be the sole source of whatever active principle can be extracted from the posterior lobe, the pars nervosa is merely the carrier for the secretory product. This is assumed to find its way in the loose tissue toward the tuberal nuclei, and the broken-up appearance of the ependymal cuticle of the infundibulum [12] strongly suggests its partial passage into the cavity of the ventricle.

* In a small selected group of forty-two imbeciles and idiots, in whose study Dr. Southard was particularly interested, thirteen (or 31 per cent) showed marked invasion. The ages ranged from 24 to 72 years, many of the patients having been institutionalized for a long period of years.

Whether the secretion of the pars intermedia under variable stimuli is capable of being chemically altered, or whether its pharmacological action can be *qualitatively* modified during its transit through the nervous tissue, is now impossible to say. But there can be little doubt that under different physiological stresses or differing conditions of disease it is *quantitatively* variable, the degree of basophilia, as already indicated, being looked upon as a measure of posterior lobe activity.

Granted that a few invading basophils may normally be found in every gland that is completely studied, how rapidly their number may multiply under proper stimulation is unknown. Karplus and Peczenik,[24] to be sure, have shown that electrical excitation of the tuber will promptly increase the amount of a posterior lobe-like substance in the ventricular fluids. But whether such a stimulus long continued would actually lead to histological changes indicating activation of the pars intermedia does not appear to have been put to the test.

From Cannon's experiments it is known that the adrenal medulla may be quickly activated and there is no reason to believe that the response of the neurohypophysis to an electrical or emotional stimulation would be any less slow. In the case of the adrenal glands, however, we do not yet know just where to look microscopically for the cytological source of the pressor principle, whereas in the neurohypophysis we apparently now do.

The several pathologists whose opinion has been consulted in regard to these matters have mostly raised the objection that a posterior lobe basophilia may occasionally be encountered in supposedly normal glands. Professor W. G. MacCallum and Professor H. M. Turnbull have both sent me sections from the pituitary bodies of persons who have died in consequence of accidents, the glands showing (Figs. 33 and 34) as rich a basophilic invasion as was present in some of the cases of eclampsia herein described.

Just what form of neurohumoral stimulation calls forth the basophilic invasion in the first place is undetermined. But it is known that the posterior lobe receives a richly arborized, non-myelinated nerve supply from the anterior hypothalamic nuclei and its functional activity is probably controlled by a diencephalic mechanism that is highly sensitive to the primitive emotions. And if, as Cannon has shown, the sympathico-adrenal apparatus can be discharged

by fright, there is every reason to suppose that the neurohypophysis is just as likely, if not more likely, to respond to crude stimuli of similar kind.

That the pars intermedia cells, under profound or prolonged nervous impulses, can multiply and ripen with sufficient rapidity to invade the lobe and discharge their secretion so as to produce in the course of a few hours the pathological picture under discussion may be assuming too much. Granting that there was no preëxisting hypertension of which the postmortem examinations gave no evidence, and being unaware of how long the patients survived, this is the only possible present explanation to offer for the basophilic infiltration of the posterior lobe in these fatal accident cases. However this may be, and some better explanation may be forthcoming, it is the purpose of this paper to offer an interpretation of those instances of posterior lobe basophilia that are associated with a *known* disorder, rather than to attempt an explanation for all conditions in which a similar process is found to occur.

3. Posterior Lobe Secretion and the Invading Elements: What are these basophilic elements that are taken to be activators of the posterior lobe, and what is their relation to the basophilic cells of the pars distalis? From the fact that in the case of pituitary basophilism not only was there a basophilic adenoma of the pars distalis but at the same time a marked invasion of the posterior lobe, it might be assumed that the elements in both regions had been simultaneously affected by the same stimulus, whatever it might be. A wholly similar dual basophilia affecting both lobes has also characterized some of the eclamptic glands that have been studied. Histological similarity, however, does not necessarily imply that the chemical nature of the secretory product of the cells is identical. While loth to get entangled in the highly controversial subject of the relation of the anterior pituitary-like substance, prolan, to the actual gonadotropic hormone of the anterior hypophysis, something nevertheless must be said regarding it in connection with the subject in hand.

Emphasis up to this point has been laid on hypertension as a manifestation of the posterior lobe activation, rather than on other less striking and less easily measurable symptoms, but this does not mean that other effects, such for example as disturbances of carbohydrate and fat metabolism, which are equally well ascribable to posterior lobe over-activity, may not at the same time be produced. The associa-

tion of diabetes mellitus with adiposity and subsequent hypertension has long been appreciated in the clinic and the suspicion of a concomitant (possibly primary) pituitary disorder been aroused. That all three of these conditions are striking features of pituitary basophilism can scarcely fail to be of significance.

That the posterior lobe might contain a gonadotropic substance, however, would scarcely be expected. Pighini [35] (1932) has reported that extracts of the human anterior hypophysis and tuber, as well as the cerebrospinal fluid from the third ventricle, give positive Aschheim-Zondek tests in immature rats. There would, however, be no way of telling whether the gonadotropic substance in tuber and cerebrospinal fluid had been transported from pars distalis by the hypophysio-portal veins or whether it had come from the pars intermedia. To this question with great profit Zondek and his collaborators have recently turned their attention.

One of the well known properties of posterior lobe extracts obtained from the glands of animals is its melanophore-expanding capacity when tested on batrachians. While the posterior lobe hormone or hormones are not normally present in sufficient amounts in the blood to be definitely detectable it had, however, been observed by Küstner, by Ehrhardt, by Dietel and others, that a melanophore-expanding substance appears in the blood serum of pregnancy and can be found in high concentration in the serum of eclamptics.

Zondek and Krohn [48] a year ago (1932), after a series of ingenious experiments in which the European minnow was used as a highly satisfactory test object for the melanophore reaction, announced that the juxtaneural strip of both the human and bovine hypophysis contains an excess of this component of posterior lobe extracts which is neither detectable in the pars distalis nor in the remote portions of the pars nervosa. The active substance, which was called "intermedin," can be traced through the stalk and the tuber, and it is demonstrable in small amounts in the fluid content of the third ventricle, though not elsewhere in the cerebrospinal fluid spaces.

Thus one at least of the constituent properties of posterior lobe extracts has been shown to be more highly concentrated in the zone of the pars intermedia from which it is in all certainty elaborated. But Zondek has gone still further and in the present year (1933) has shown [47] that in the human (but not in the bovine) hypophysis a sex-

maturing substance identical with prolan A is present in this same strip of posterior lobe which lies adjacent to its epithelial investment. Traces of it are also found in the stalk but not in the third ventricle, in which respect it differs from intermedin. Under the influence of Berblinger, Zondek concludes that this substance represents the inwandering basophils from the pars distalis (*sic*). Prolan, he believes, must therefore be derived from the basophilic elements of the anterior lobe.

From what source the human glands used in these experiments by Zondek were obtained and to what maladies the subjects may have succumbed is not mentioned. Nor could the tissues have been used both for the making of an extract and for the histological demonstration or otherwise of an active posterior lobe basophilia. It is quite probable, however, that had the posterior lobe activation by basophils in these glands been sufficiently marked, the sex-maturing substance might also have been demonstrable in the fluid of the third ventricle. Hints suggesting this possibility have been provided from another source, namely, from the studies by certain gynaecologists. The evidence at hand has been summarized briefly as follows by Eugen Kulka.[29]

Aschheim, in searching for follicle-ripening substances in various fluids and tissues of pregnant women, failed to find any trace of such a substance in the cerebrospinal fluid. Califonza, on the other hand, believed that he had detected its presence in fifteen out of the twenty-eight fluids examined. Ehrhardt [16] found prolan A in the cerebrospinal fluid in three cases of eclampsia, in one preëclamptic, and in a gravid woman suffering from carcinoma; and Heim states briefly that he had corroborated these findings in eclamptics. Kulka investigated the lumbar fluid from twenty-five gravid patients, seven of them with symptoms of marked eclampsia. The Aschheim-Zondek test was negative in the fluid in all but six of the patients. Of the six cases showing a positive reaction one had intra partum eclampsia with a blood pressure of 190, labour having been induced by forceps. Another was a postpartum eclamptic with a blood pressure of 200, oedema of the extremities and albumin in the urine. The third patient had a cystic chorionepithelioma and three others were examples of marked hyperemesis gravidarum.

While the evidence given by these several writers is suggestive rather than conclusive, it is remarkable that under any circum-

stances of posterior lobe activation an active principle should be found in the cerebrospinal fluid obtained by lumbar puncture. Could the fluid from the ventricles have been examined, or even that from the posterior cistern, the chance of detecting the substance looked for would have been vastly greater.

More important are the recent biochemical studies by Anselmino and his collaborators, to which allusion has already been made. In their more recent paper [2] (1932) it is claimed that the active substances found in the blood of eclamptics are identical with the corresponding fractions of posterior lobe extract and that their amount varies quantitatively with the severity of the symptoms. They assume that the combination of excessive pressor and antidiuretic effects leads to arteriolar and capillary spasm with water retention and oedema of the tissues. When the brain becomes oedematous convulsions and coma are produced and there is usually a terminal oedema of the lungs. They believe that overproduction of the posterior pituitary hormone affords the only consistent explanation of these phenomena. All this seems the more plausible in view of certain observations such as those by Rowntree,[39] by Dietel,[15] and by McQuarrie and Peeler [32] on the clinical and pathological consequences of experimental water intoxication, whether produced by administering excessive amounts of water or by the antidiuretic effect of posterior pituitary extracts.*

While the studies mentioned above are highly suggestive, they are concerned with some of the better known properties of posterior lobe extracts and have no apparent bearing on the possible production by the posterior lobe of the sex-maturing substance that Zondek has found to be present in the juxtaneural portion of human glands. In this connection the following observations would seem to be of great significance.

Drs. G. Van S. and O. W. Smith of Boston have recently shown [43] that the blood and urine of toxaemic patients in late pregnancy contain a far larger amount of the anterior pituitary-like substance (prolan) than ordinarily occurs in pregnancy. They have further demonstrated [44] in a second communication that a quantitative imbalance between prolan and oestrin is characteristic of the

* Efforts to produce in animals lesions in the liver and kidneys comparable to those characterizing human eclampsia by administering posterior lobe extracts have been highly contradictory (*e. g.*, the papers by Dietel,[16] by Fauvet,[19] and by Ohligmacher [33]).

toxaemias of late pregnancy. The number of rat units per 100 cc. of blood serum in twelve gravid women without symptoms averaged 50, in eighteen toxaemic patients 250, and in five eclamptics 480. The amount of oestrin was correspondingly diminished. In the course of this study the interesting observation was made on a gravid woman with diabetes insipidus that the amount of pituitrin necessary to control the polyuria was greatly diminished during the months of child-bearing.

It can be gathered from all this that information from many sources points toward a hyperactivation of the posterior lobe in these hypertensive states. And if we are to believe, as some of the observations strongly suggest, that prolan is a product of posterior lobe basophilia, while the gonadotropic substance extracted from the anterior lobe is derived from the basophils of that part of the gland, the difference in the reactions of these two sex-maturing substances, which so many have pointed out, may thus be accounted for.

Summary and Conclusions

The active principle of the posterior lobe and its several fractions must under all circumstances primarily be derived not from the pars nervosa but from its epithelial investment — the pars intermedia.

When the posterior lobe of man is functionally dormant the pars intermedia is inconspicuous, but so soon as it is activated the investing cells become transformed into basophilic elements, which in certain areas invade the pars nervosa. When their cytoplasm becomes fully ripened the cells eventually lose their staining qualities, change first into discernible "hyaline bodies" and then into a fluid product, which apparently makes its way through the loose tissue spaces of stalk and tuber in the direction of the infundibular ventricle and the adjacent hypothalamic nuclei.

Under certain circumstances the invading basophils with their desquamated products are greatly increased in number and the cellular infiltration assumes a massive character. This is looked upon merely as a pathological exaggeration of the normal secretory process and its degree is regarded as a measure of the hyperactivation.

An extreme example of posterior lobe basophilia of this sort has been observed in a case of so-called "pituitary basophilism," associated with a functionally active basophilic adenoma of the pars distalis. This polyglandular disorder chiefly affects young persons and

is characterized, among other symptoms, by vascular hypertension together with disturbances of carbohydrate and fat metabolism. As these symptoms suggest a posterior rather than an anterior lobe effect, it was assumed that the posterior lobe basophilia represented something more than an overflow of these elements from the anterior lobe.

It has been shown by Anselmino and his collaborators that the blood of eclamptics with oedema and marked hypertension contains antidiuretic and pressor substances, whose effects correspond to those produced by posterior lobe extracts. They therefore claim to have proved what others had suggested, that the toxaemias of pregnancy were due to the overproduction of the posterior lobe hormones.

In serial sections of six out of nine pituitary bodies from fatal cases of eclampsia a heavy infiltration of basophilic elements in the posterior lobe has been disclosed, and the same condition has been observed in a number of glands from cases of essential or nephrovascular hypertension, also serially cut and examined. That in advancing years there is a tendency for the basophilic cells thus to wander in large numbers into the posterior lobe has long been known. It has been looked upon merely as a concomitant of old age, particularly when attended by atherosclerosis and renal disease.

Pathologists have recognized in eclampsia distinctive lesions in the liver to which the disorder has customarily been ascribed. In essential hypertension, likewise, lesions affecting the terminal arterioles of the kidneys have been thought to indicate a primary nephrovascular disorder. Necroses in eclampsia, however, are not limited to the liver, nor are the terminal arteriolar lesions in essential hypertension confined to the kidneys. In neither instance do the histopathological findings satisfactorily account for the clinical symptoms.

From the observations presented the conclusions are drawn: (1) that the source of these hypertensive disorders lies in the posterior lobe of the pituitary body; (2) that the extent of basophilic invasion from the pars intermedia is a measure of posterior lobe activity; and (3) that excessive infiltration by these elements represents the histopathological basis of eclampsia and essential hypertension in young persons and may possibly also be related aetiologically to the atherosclerosis of old age.

Whether the general hypothesis herein advanced should or should not prove on further study to be in all its features wholly correct, it will nevertheless provide an incentive to include a detailed study of the neurohypophysis in forthcoming postmortem studies of disorders in which hypertension is a distinguishing feature.

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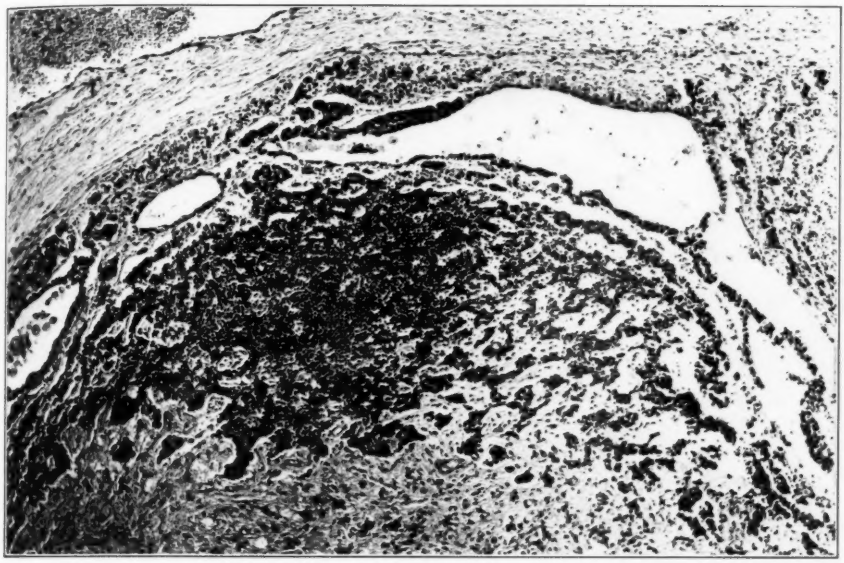
DESCRIPTION OF PLATES

PLATE 54

FIG. 1. Massive posterior lobe invasion from a case of pituitary basophilism (mag. × 60).

FIG. 2. Section (mag. × 9) from the Raab-Kraus case of basophilic adenoma presumably arising from pars intermedia (*cf.* Fig. 3).



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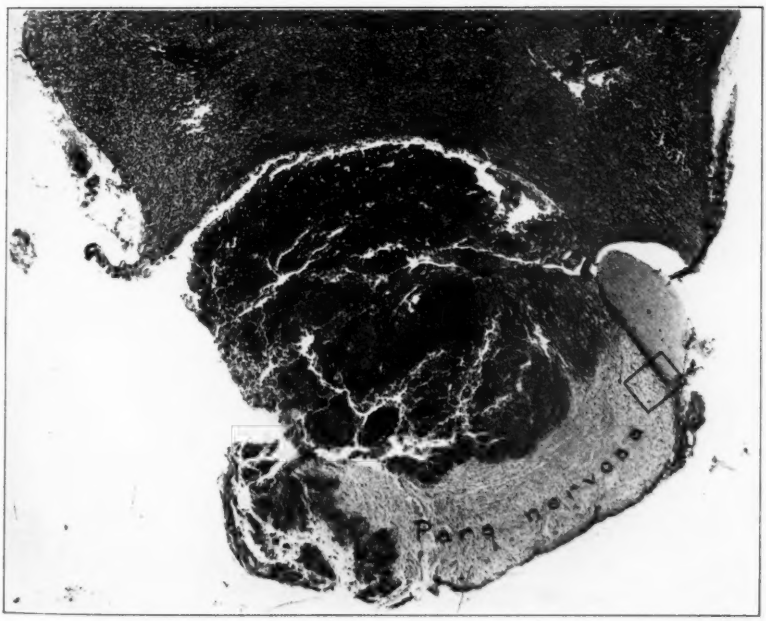
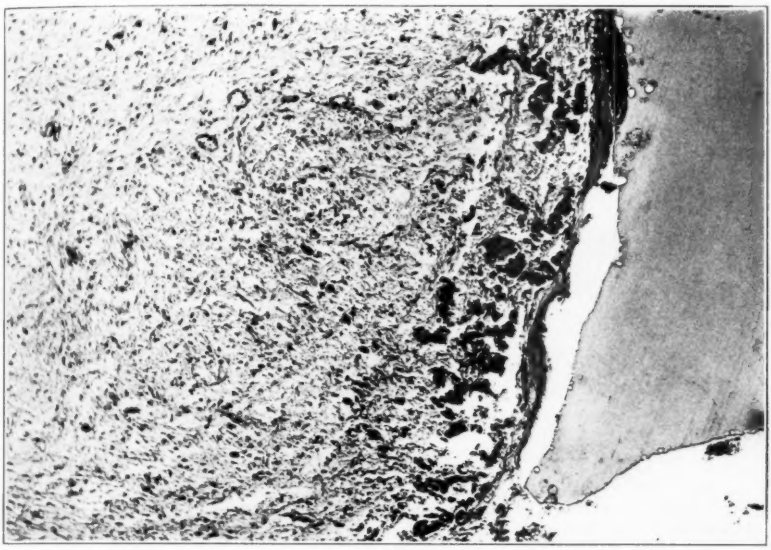


2

PLATE 55

FIG. 3. Squared area from Fig. 2 (mag. × 80) showing infiltrating basophils in a zone remote from the adenoma.

FIG. 4. Typical cone-shaped area of basophilic invasion from outer angle of pars intermedia in a case of presumed eclampsia (mag. × 80).



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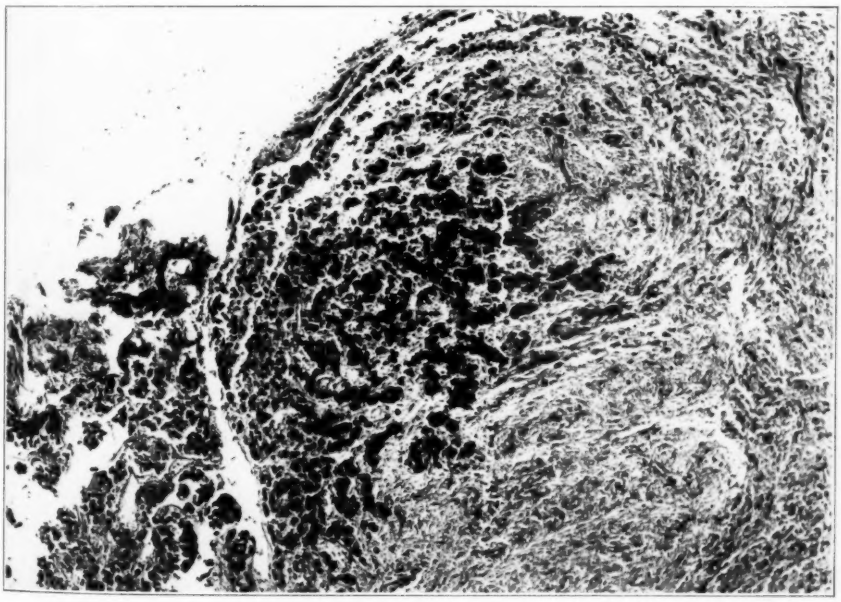


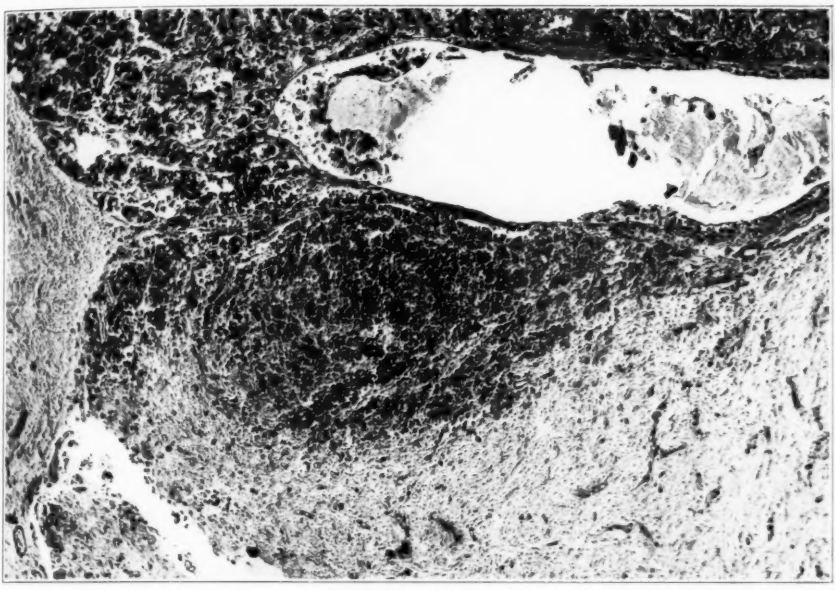
4

Cushing Hyperactivation of the Neurohypophysis

PLATE 56

FIG. 5. (Case 2.) Posterior lobe infiltration by basophils from a case of eclampsia with hypertension (mag. × 60).

FIG. 6. (Case 2.) Showing (mag. × 300) in center of pars nervosa accumulations of hyaline masses (Herring) in the spaces that are bounded by the "baskets" of neurofibrils.



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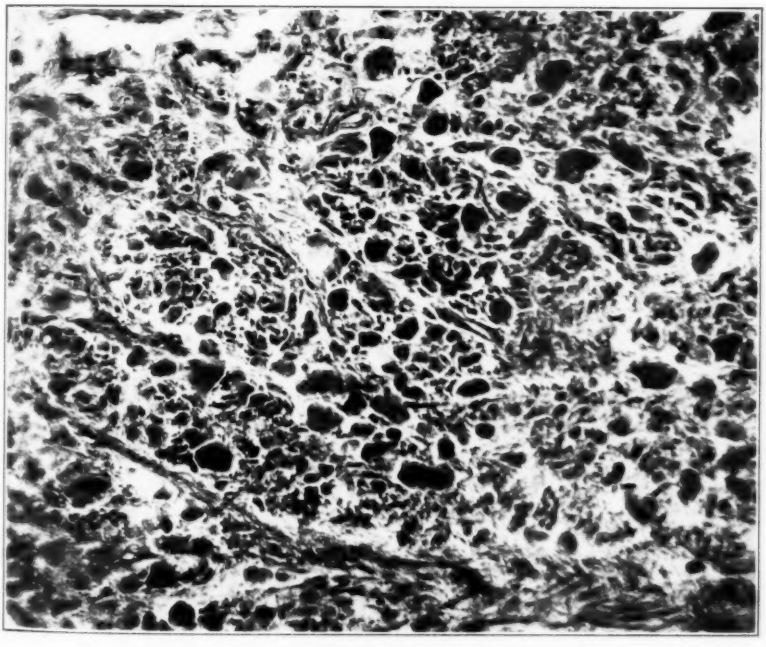

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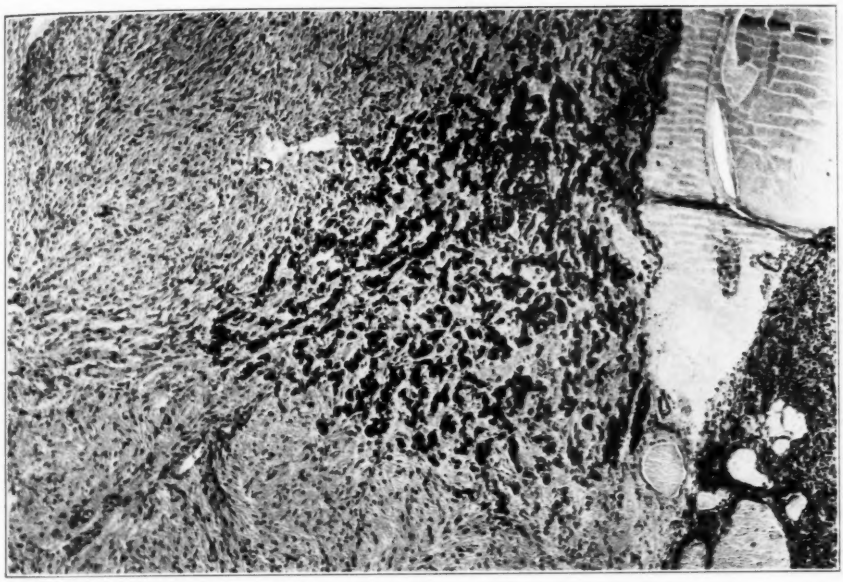
PLATE 57

FIG. 7. (Case 3.) Invasion of basophilic elements from pars intermedia in a case of eclampsia. Note separation from pars anterior (upper left) by large colloidal mass reopening residual cleft (mag. × 60).

FIG. 8. (Case 3.) Showing (mag. × 560) masses of granular holocrine secretion in and among tongues of invading basophilic elements.



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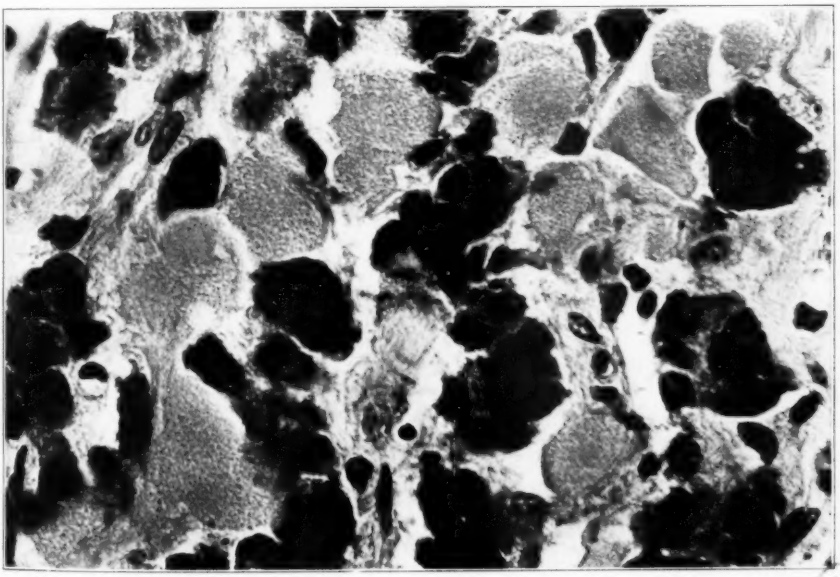

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PLATE 58

FIG. 9. (Case 4.) Posterior view of block of tissue with large gland, bulging posterior lobe and juicy tuber in case of eclampsia.

FIG. 10. (Case 4.) Horizontal section (Section 1260) through lower portion of posterior lobe to show widespread encirclement by actively invading basophils (mag. × 20).

FIG. 11. (Case 4.) Showing area (mag. × 60) squared in Fig. 10.

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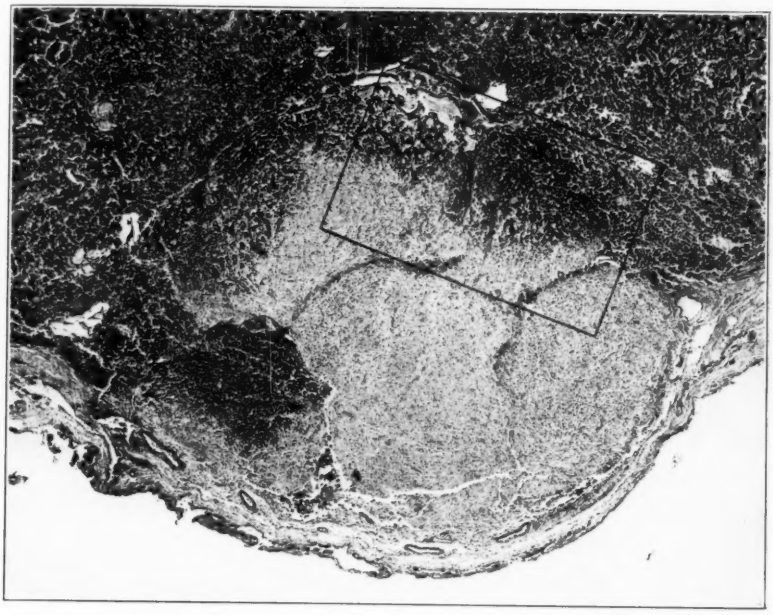

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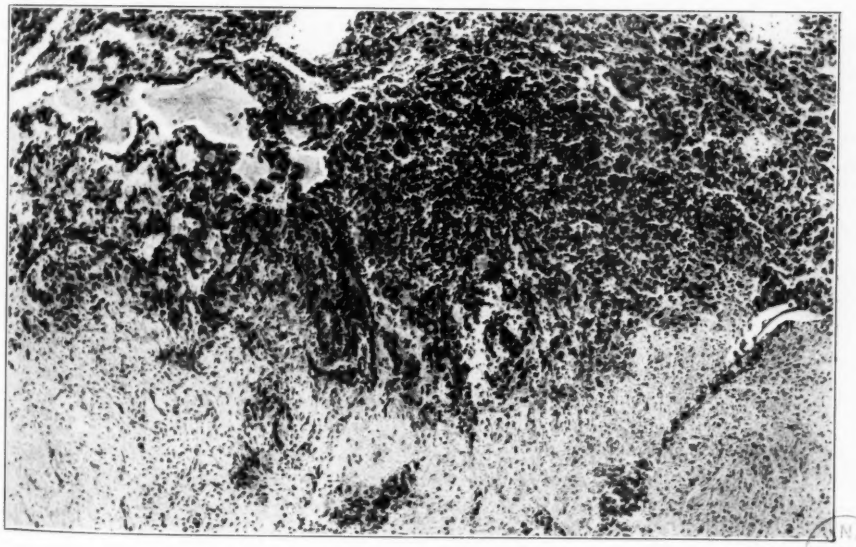

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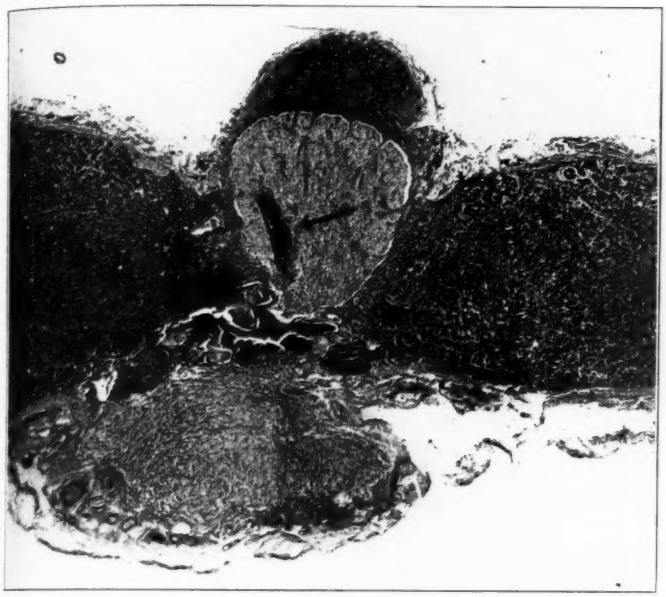
PLATE 59

FIG. 12. (Case 4.) Showing (Section 3060, mag. × 15) strand of basophils (arrow) in lower stalk. At this level through upper part of gland the pars tuberalis showing above has just become free from pars distalis. Insert (Fig. 12a) shows (Section 3510, mag. × 15) same strand of viable cells still traceable in free stalk.

FIG. 13. (Case 4.) To show (mag. × 600) the holocrine discharge of ripened cells between invading elements. Note ghosts of nuclei in several of the secretory masses.



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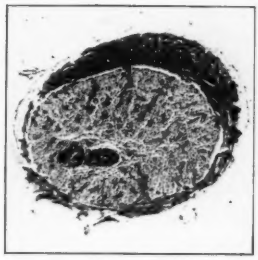

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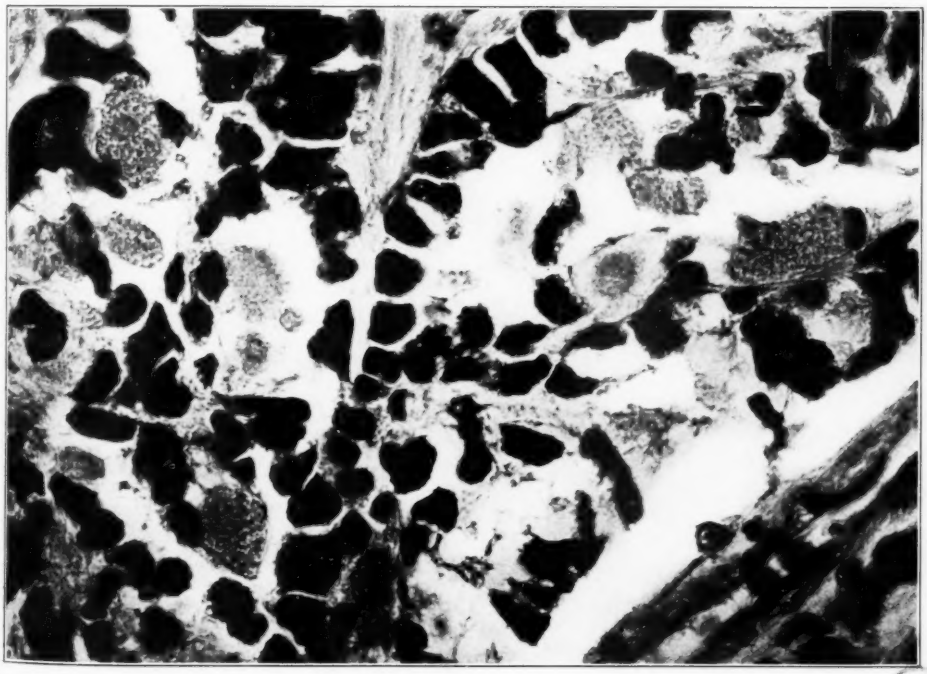

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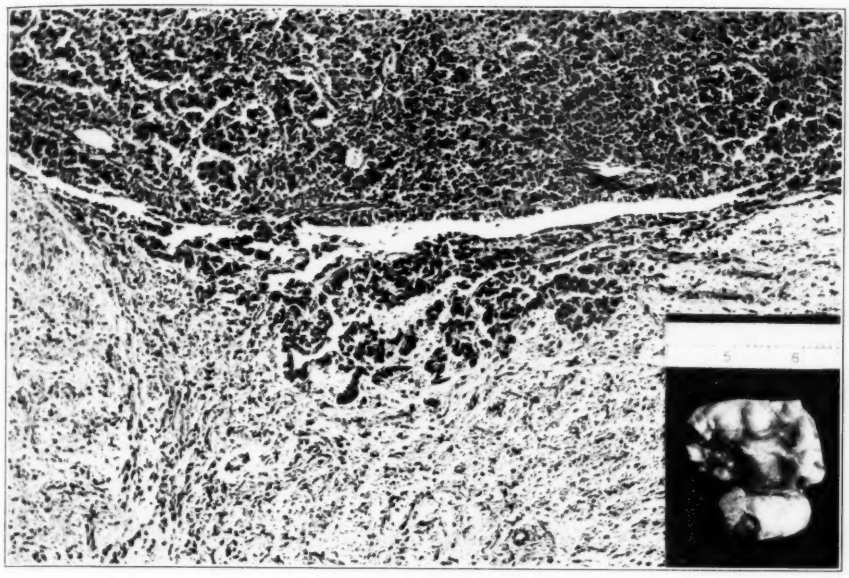
Plate 60

Fig. 14. (Case 5.) Showing (mag. × 80) one of two areas of moderate conical infiltration traceable in other sections into center of pars nervosa. Insert shows the posterior view of the specimen (natural size) with pituitary body below.

Fig. 15. (Case 5.) Showing (mag. × 230) pars intermedia activity with formation of Rathke's cysts lined by ripened basophils. Pars distalis (above) separated by cleft from posterior lobe (below).



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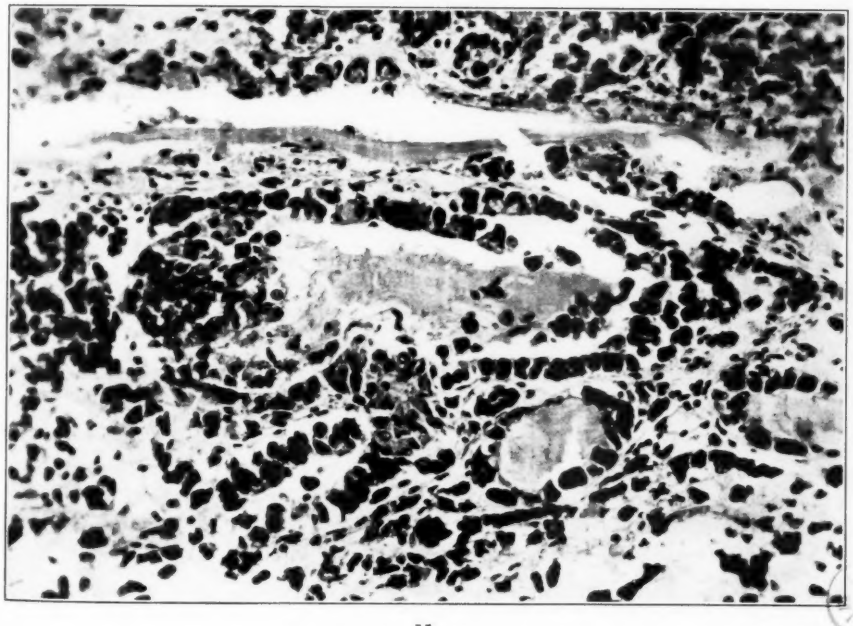

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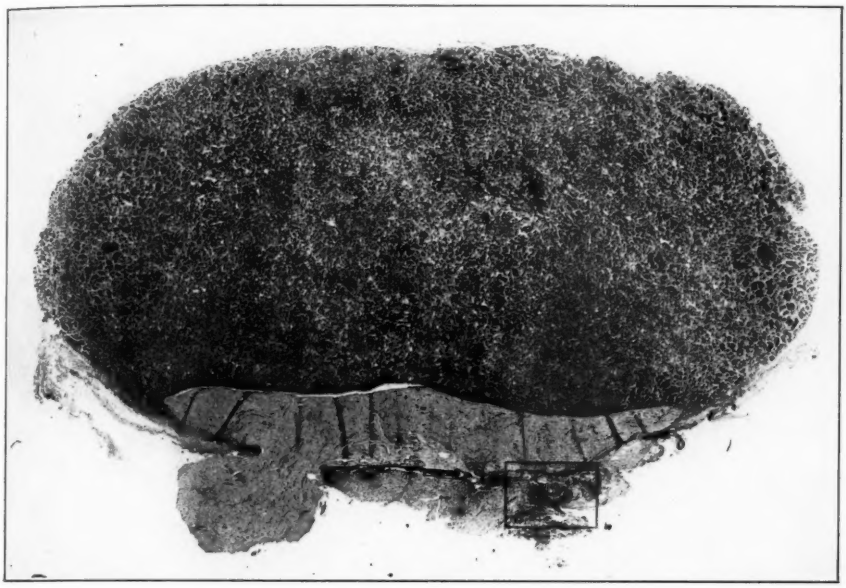
Cushing Hyperactivation of the Neurohypophysis

Plate 61

Fig. 16. (Case 6.) Section 630 (mag. × 8) showing large anterior lobe with entire cleft distended by colloid. Posterior lobe somewhat damaged in removal. Basophilic invasion from pars intermedia in squared area.

Fig. 17. (Case 6.) Squared area from above (mag. × 70) showing invasion from pars intermedia.



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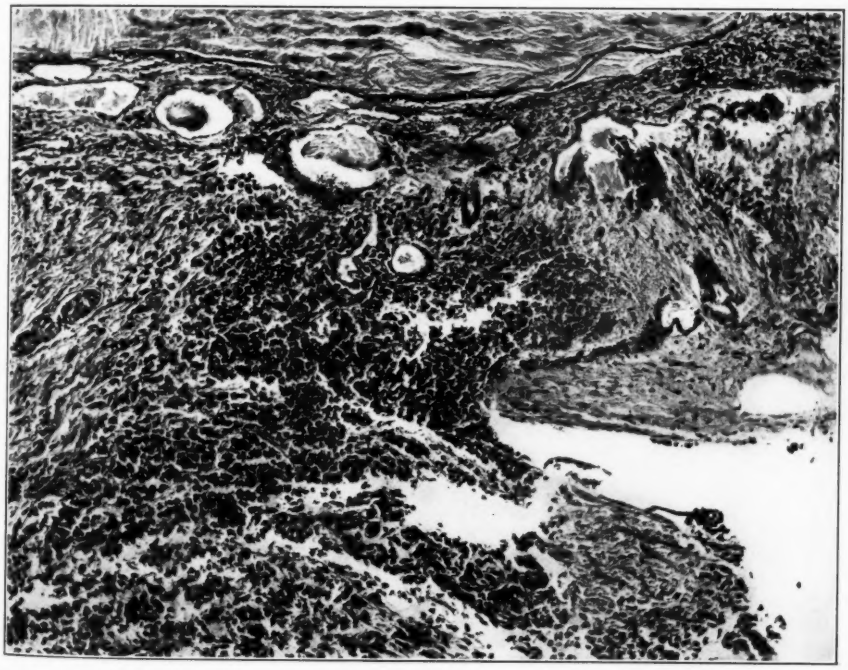

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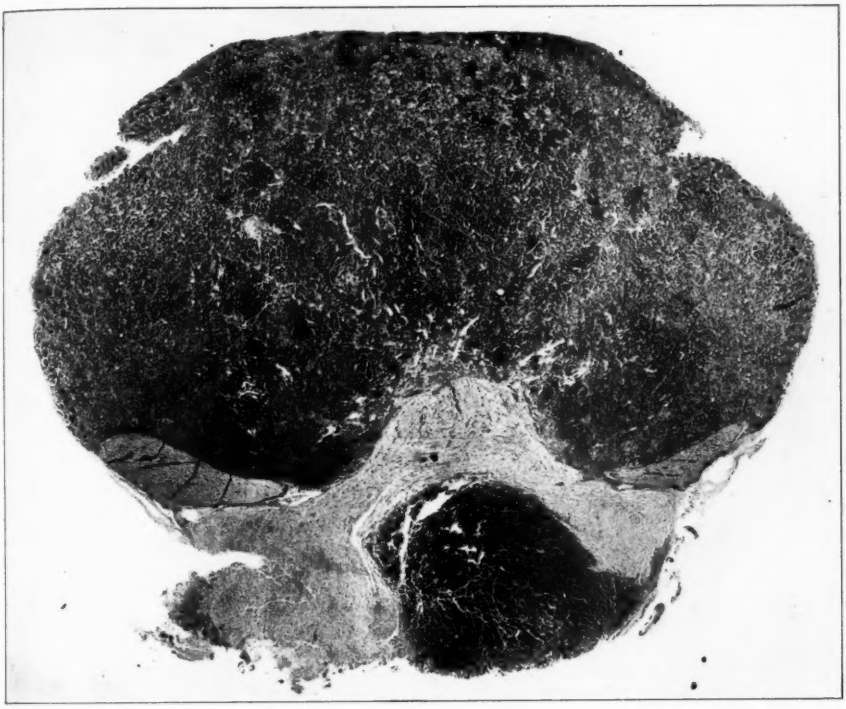
PLATE 62

FIG. 18. (Case 6.) Section 1710 (mag. × 8) taken at level where stalk of posterior lobe is forming and large portal sinusoids are congregating toward it. Note large basophilic adenoma in posterior lobe.

FIG. 19. (Case 6.) Section 2250 (mag. × 8) showing adenoma fading off at posterior edge of pars nervosa. At this level the pituitary stalk has already formed and the portal vessels are clearly shown radiating backward toward it in what will become pars tuberalis.



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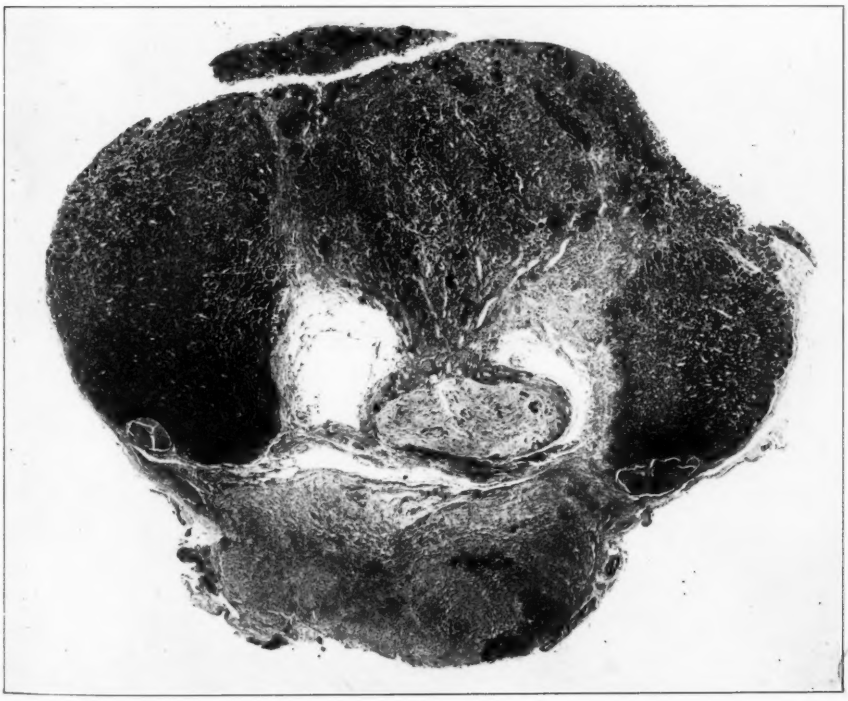

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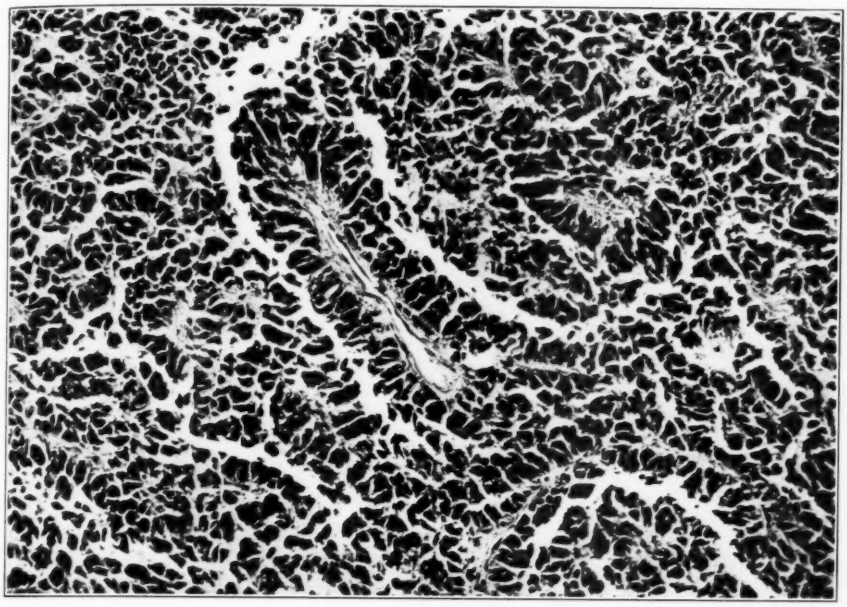
PLATE 63

FIG. 20. (Case 6.) To show (mag. × 150) general character of adenoma whose cells bud off from capillary stalks.

FIG. 21. (Case 6.) Showing on higher magnification (× 600) the typically vacuolated basophilic elements of the adenoma.



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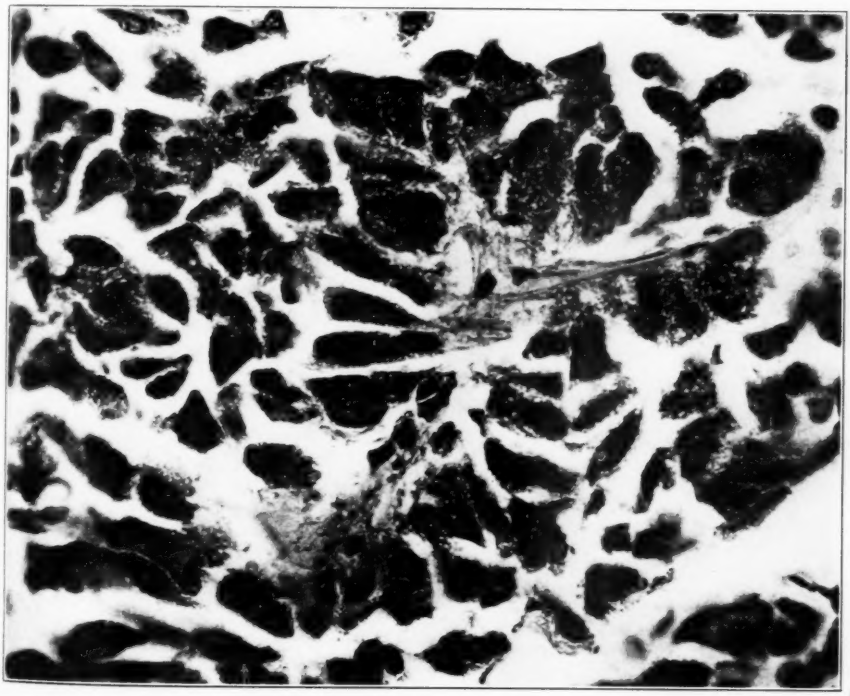

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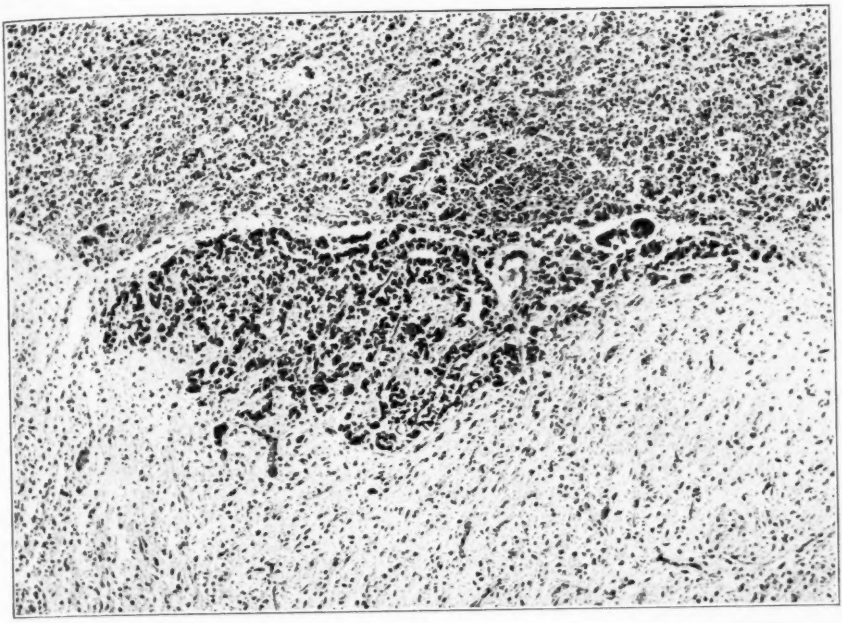
Cushing Hyperactivation of the Neurohypophysis

PLATE 64

FIGS. 22 and 23. Showing (mag. × 80) the relatively slight degree of invasion in Case 7 (above) and Case 8 (below).



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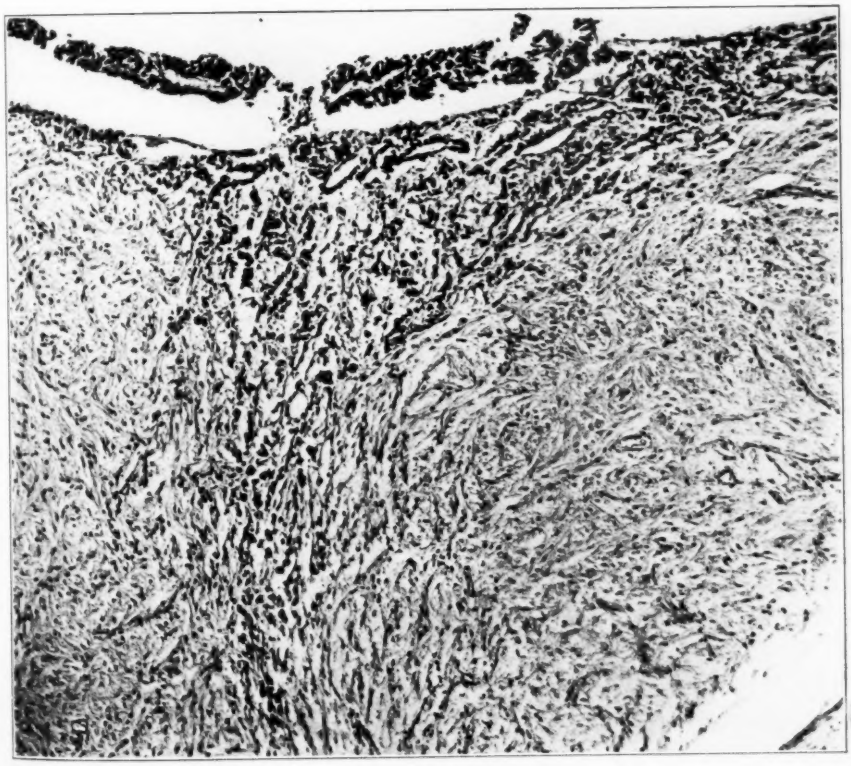


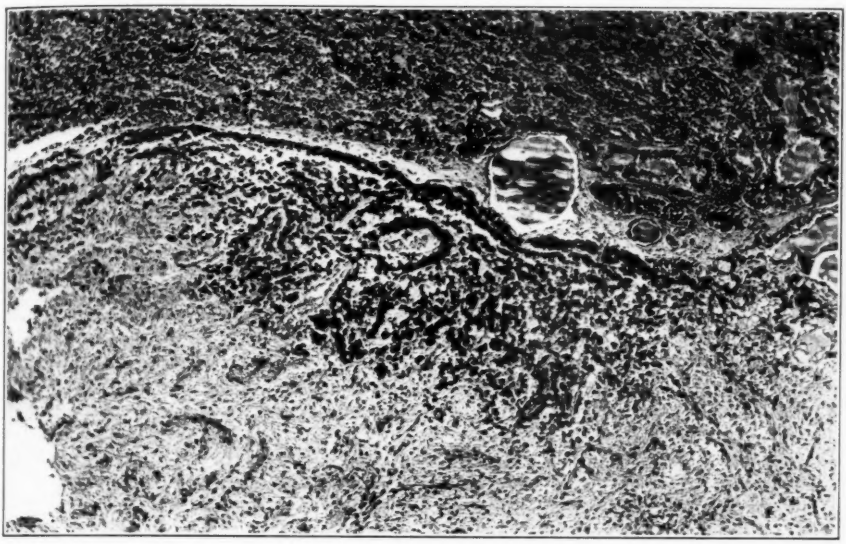
23

Cushing Hyperactivation of the Neurohypophysis

Plate 65

Fig. 24. (Case 10.) Zone of activated basophils from pars intermedia from a case of essential hypertension (mag. × 60) in a man of middle age.

Fig. 25. (Case 11.) Showing (mag. × 60) posterior lobe invasion in a 60 year old man with hypertension and atherosclerosis.



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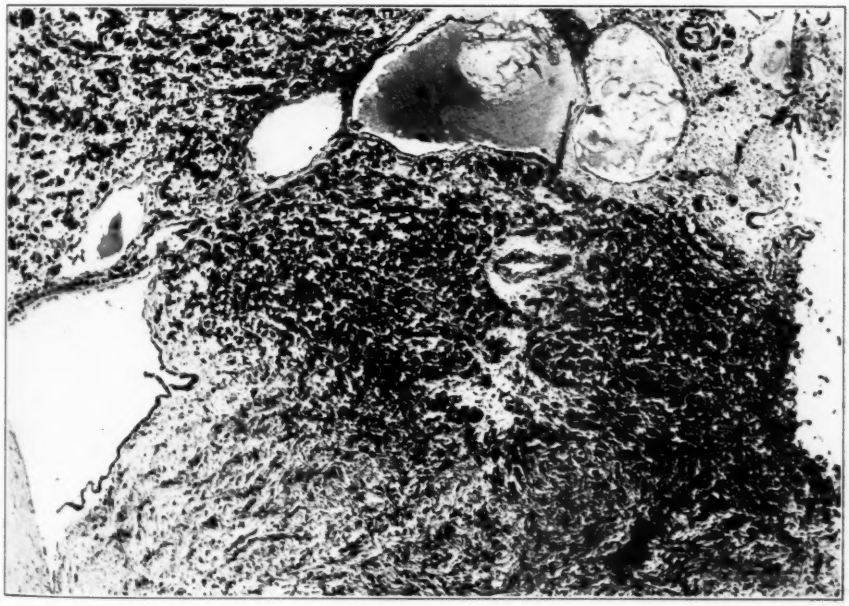
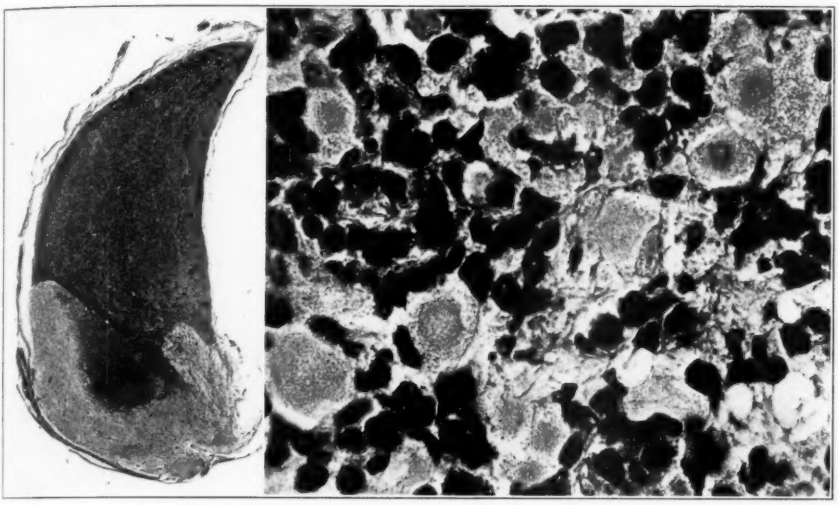


25

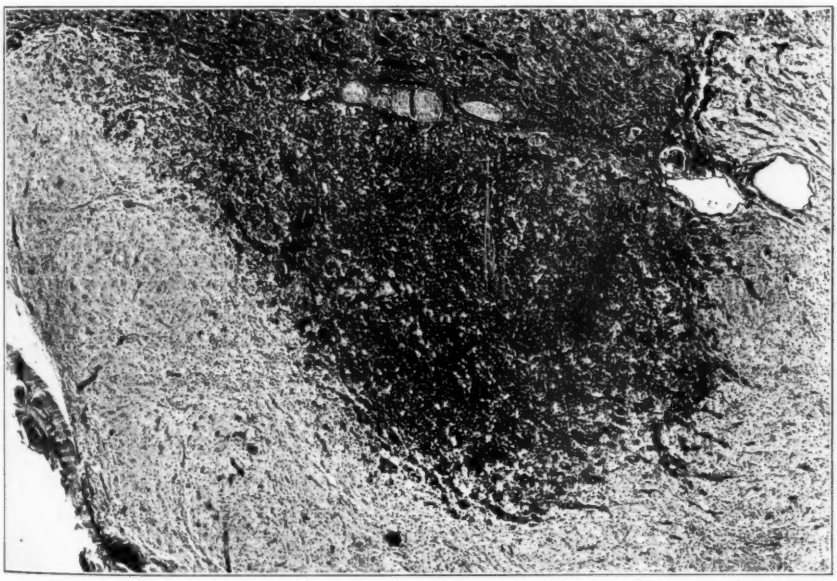
Plate 66

Figs. 26 and 27. (Case 12.) Sagittal section (mag. × 8) of small gland from 67 year old woman with marked hypertension and heavy posterior lobe invasion. In Fig. 27 (mag. × 600) are seen well preserved masses of holocrine secretion showing ghosts of swollen nuclei.

Fig. 28. (Case 12.) Showing (mag. × 40) the area of massive invasion easily visible to the unaided eye (*cf.* Fig. 26).



26 27

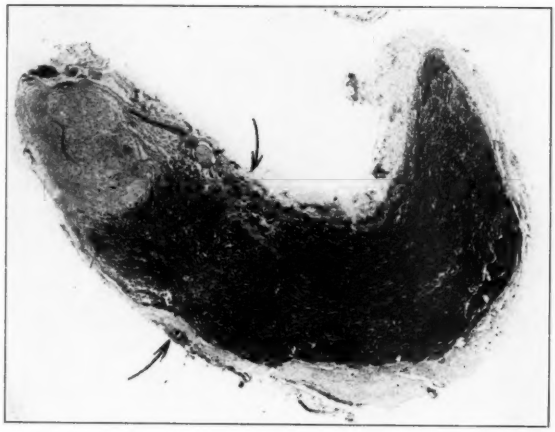


28

Plate 67

Fig. 29. (Case 13.) Sagittal section (mag. × 8) from gland of an aged woman with atherosclerosis, showing massive posterior lobe invasion. Arrows point to position of cleft.

Fig. 30. (Case 13.) Showing on higher magnification (× 30) the full extent of the infiltration. A corner of pars distalis shows in the lower left corner.



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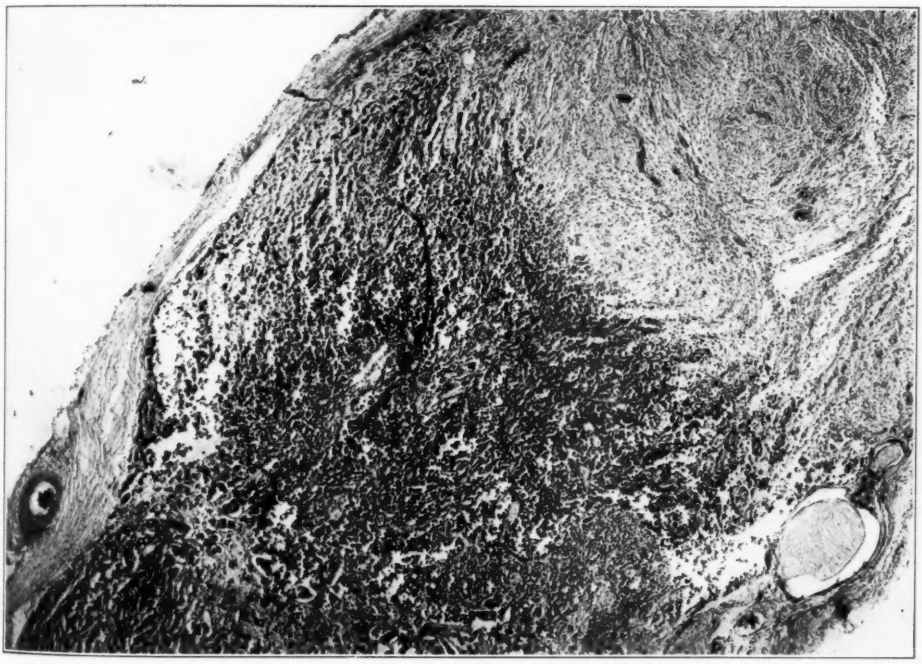
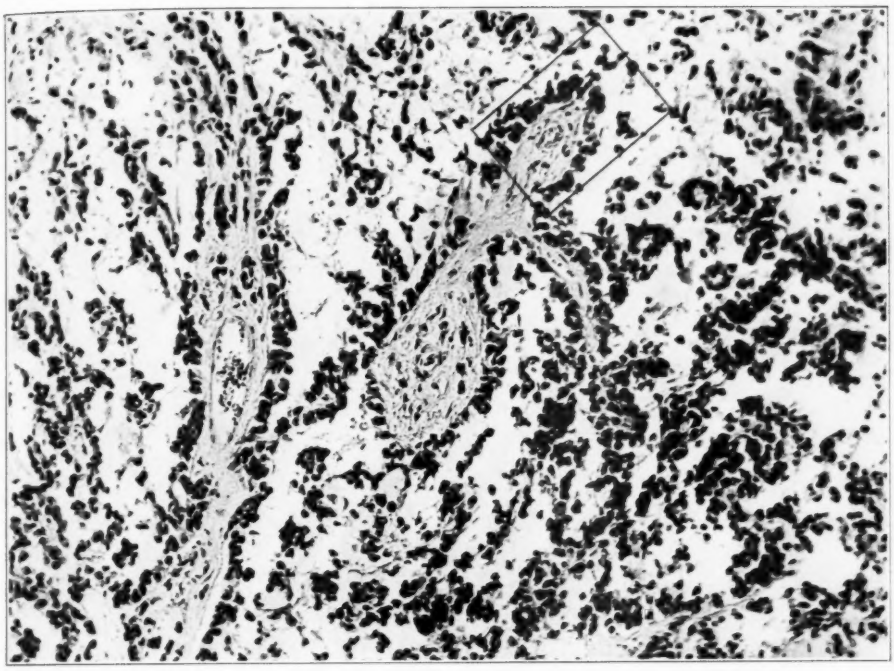


30

PLATE 68

FIG. 31. (Case 13.) Posterior fringe of invading elements.

FIG. 32. (Case 13.) Squared area from Fig. 31 (mag. × 850) to show typical vacuolated cytoplasm of basophilic elements.



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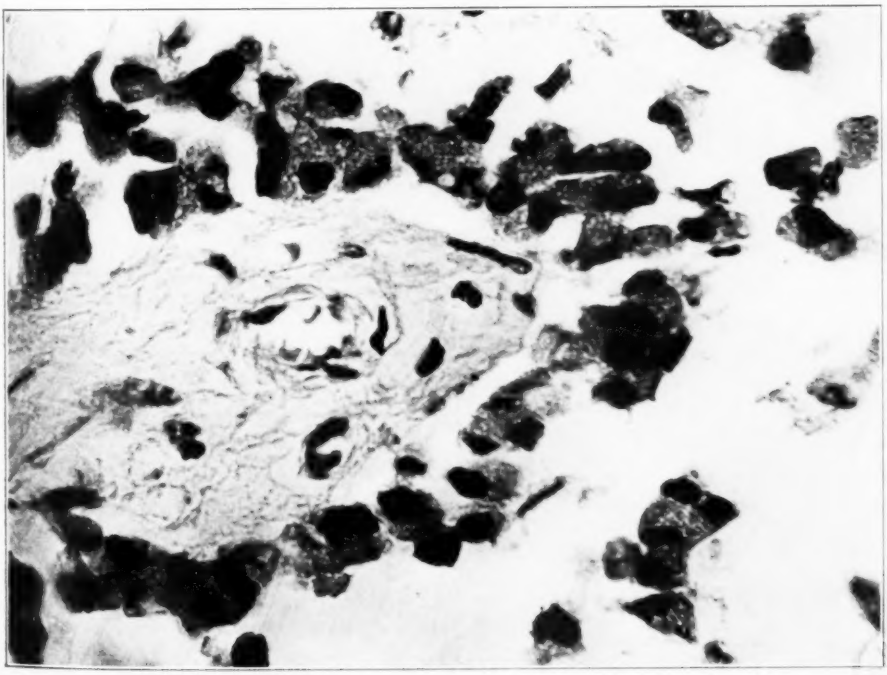
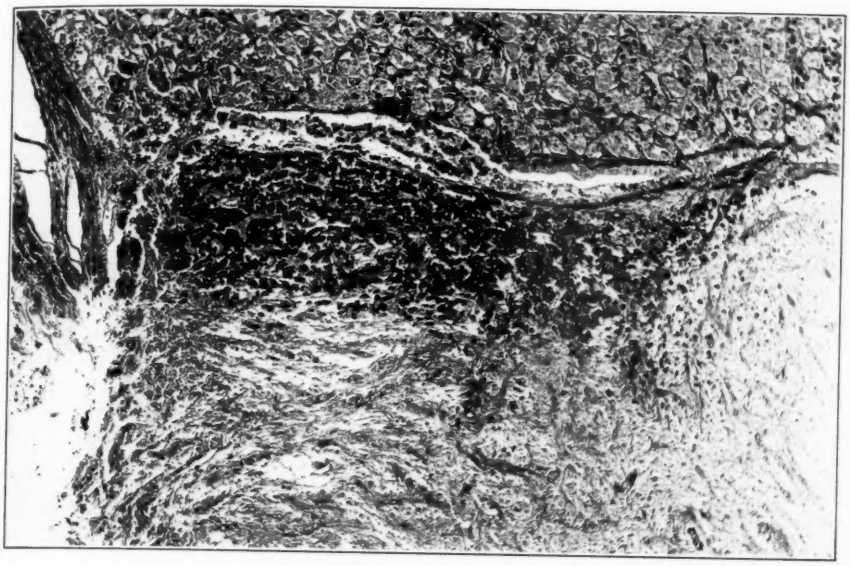


32

Cushing Hyperactivation of the Neurohypophysis

PLATE 69

FIGS. 33 and 34. Moderate invasion (× 60) in two cases of accidental death. (Kindness of Professor Turnbull.)



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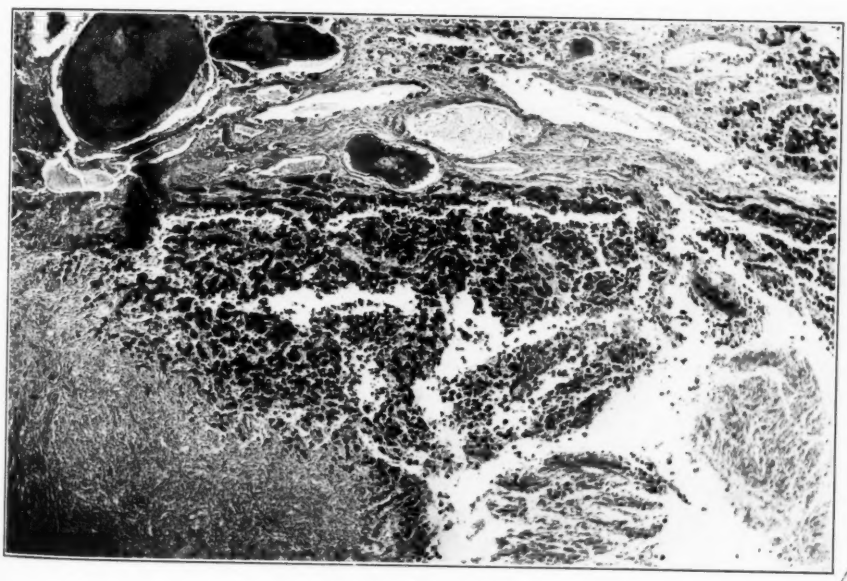

34

CARDIOVASCULAR RENAL CHANGES ASSOCIATED WITH BASOPHIL ADENOMA OF THE ANTERIOR LOBE OF THE PITUITARY (CUSHING'S SYNDROME)*

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(From the Pathological Departments of Tufts College Medical School, Boston, Mass., Guys Hospital, London, England, and the Peter Bent Brigham Hospital, Boston, Mass.)

This brief report is submitted through the kindness and encouragement of Dr. Cushing of the surgical department of the Peter Bent Brigham Hospital in Boston, and Drs. Bishop and Close of the pathological department of Guys Hospital in London, who have allowed us to make histological studies of the kidneys and other organs from patients showing clinical signs of pituitary basophilism.

When one groups together the cases reported of basophil adenoma of the pituitary in order to study and to unravel the complex clinical syndrome one finds recurring with a marked regularity certain signs and symptoms indicative of cardiovascular renal pathology. Emphasis has already been focused on this point by Cushing [1, 2, 3] and others, but we can find no mention in the literature as to just what type of cardiovascular renal pathology occurs in such cases. Is it possible that it is merely a coincidence that a cardiovascular renal picture should be found in patients showing this rather rare disease, or are we dealing here with a cardiovascular renal problem that is definitely an intrinsic part of the syndrome of pituitary basophilism?

In a group of patients showing pituitary basophilism, recently reported by Cushing, none had passed middle life but the blood pressure, both systolic and diastolic, was elevated and at times associated with headache, blurring of vision and retinal hemorrhages. In many cases years passed before signs and symptoms suggesting renal pathology appeared, and then they were sometimes transitory and variable. A clinical study of the "*formes frustes*" of pituitary basophilism offers little help in identifying the nature of the cardiovascular renal lesion associated with basophil adenoma, where an elevated blood pressure together with a large heart may be the only noteworthy finding. A study of the advanced cases, on the other

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hand, offers a possible key to this solution. In such patients one may find in addition to the hypertrophy of the left ventricle and hypertension defective excretion of phenolsulphonephthalein, an elevation of the non-protein nitrogen of the blood, a fixed specific gravity of the urine, a failure to dilute and concentrate fluids or to concentrate urea when taken orally, and lastly, one may find on examination of the urine a variable amount of albumin, hyaline and cellular casts, polymorphonuclear leukocytes, desquamated epithelial cells and varying quantities of erythrocytes. Edema may be present. Such findings as these have led to the following diagnoses: chronic nephritis, vascular nephritis, and granular atrophy of the kidney.

If one disregards for a moment such symptoms of pituitary basophilism as adiposity, kyphosis, amenorrhea, hypertrichosis, a plethoric appearance of the skin, polycythemia and backache, and focuses on the cardiovascular renal problem alone, one sees at once a striking similarity to the clinical picture of malignant nephrosclerosis, as originally described in 1914 by Volhard and Fahr.[4,5] This is characterized by an elevated blood pressure, a large heart, an increase in the non-protein nitrogen in the blood, a diminution in the concentrating and diluting power of the kidney, polyuria, neuroretinitis and uremia. Edema may or may not be present. An examination of the urine in these cases will show albumin, granular, hyaline and cellular casts, polymorphonuclear leukocytes, and frequently frank blood. One may find similar signs and symptoms in chronic glomerulonephritis, but the course of both disease and the relation and sequence of symptoms to one another in chronic glomerulonephritis and malignant nephrosclerosis differ. In the latter disease, early and even in more advanced cases the cardiovascular symptoms stand far in the foreground. An elevated blood pressure and left ventricular hypertrophy that may by chronic glomerulonephritis be slight or absent are developed in patients with malignant nephrosclerosis to a remarkable degree.

In July of the summer of 1933, while visiting the pathological laboratory at Guys Hospital, London, two of us with Dr. Osman had the opportunity to study histologically tissue from a case of basophil adenoma of the anterior lobe of the pituitary, which had recently been reported by Bishop and Close.[6] To our surprise the histological picture was similar to that of malignant nephrosclerosis. On returning to America, Dr. Cushing, who has long been interested

in this same problem, allowed us to study sections from the kidney from one of his cases of basophilic adenoma of the pituitary,* and this, like the case of Bishop and Close, showed without question the histological findings of malignant nephrosclerosis.

Malignant nephrosclerosis, neither clinically nor at the autopsy table, is a common disease: in contrast to the frequency with which one meets patients with benign essential hypertension, malignant nephrosclerosis is rare. The purpose of this paper, however, is neither to describe nor to discuss in detail the clinical and histological changes of malignant nephrosclerosis, but rather to point out an extremely interesting clinical and pathological finding, namely, the presence of malignant nephrosclerosis in two patients with basophil adenoma of the anterior lobe of the pituitary.

Case Reports

Case 1. C. P. (case reported by Bishop and Close[6]), was a normal child until the age of 11 years, when she stopped growing and began to gain weight. She developed a ruddy complexion and her hair began to fall out. Menstruation began normally at the age of 14, but after a year the periods ceased, and except for 3 consecutive months, when she was 18 years of age, she suffered from amenorrhea. From the age of 14 onward she experienced severe headaches which occurred regularly every month. Six months before death the sight of the left eye became affected and 2 months later symptoms of excessive thirst and polyuria became manifest. There was also frequent backache.

At the age of 22, in November 1930, she was admitted to Guys Hospital. She was kept under observation for some time and was then discharged, but was readmitted a short time before death. On admission the most striking features were the very red complexion, dry scaly skin, hairiness of the face, chest and abdomen, and the stunted growth. A beard sufficient to necessitate the use of a razor was present. There was a patchy red erythema localized particularly to the left arm. She was slightly knock-kneed and there was a deformity of the left wrist and right hand. A radiogram showed a fissured fracture of the lower end of the left radius and a rarefaction without evidence of inflammation of the head of the fourth right metacarpal bone. While in the hospital the patient slept badly and complained mainly of thirst. Occasionally there was a feeling of suffocation. The headaches were troublesome and on many occasions the blood pressure was as high as 300 mm. Hg. After she had been in the ward for about a fortnight she had the first of a series of fits, which were relieved on three occasions by venesection, while lumbar puncture was performed about twice a week. Her intelligence was in no way impaired although her illness worried her a great deal, and she frequently resorted to tears. Her eyesight troubled her and there was a marked degree of retinitis with silver wire arteries and a scotoma of the left eye. Examination showed the heart to be slightly enlarged to the left with a loud aortic second sound. The average

* See Ref. 3, page 521.

blood pressure readings were 250/180 mm. Hg. The hemoglobin was 95 per cent. Records of the blood picture are unfortunately not available but it is believed that a red cell count was never higher than 5,000,000. The blood urea was 43 mg. per 100 cc. Blood sugar tolerance test showed delayed return to normal with a high fasting figure (0.14 gm. per 100 cc.). The blood sugar went up to 0.25 per cent and was still raised after 2 hours (0.21 per cent). The blood cholesterol was also slightly above normal (0.185 per cent instead of 0.150 per cent). The Wassermann reaction was negative. The serum calcium figure was within normal limits.

An investigation of the urine showed albumin and sugar to be present but no acetone. Pus, fatty and hyaline casts were also demonstrated. The concentrating power of the kidney was slightly defective. Shortly after the second admission to the hospital signs of acute edema of the lungs developed suddenly and death occurred.

When we examine this report and focus our attention on the cardiovascular renal problem alone, we find a young person with marked hypertension and a large hypertrophied left ventricle, suffering from headaches and disturbances in vision. An examination of the eye grounds revealed a marked degree of retinitis. The blood urea was considerably elevated and the blood cholesterol was above normal. The urine showed albumin, casts of various sorts and defective concentration. Surely from such findings our clinical diagnosis would rest between chronic glomerulonephritis and malignant nephrosclerosis, and when one considers the sequence of events the latter diagnosis is much more likely.

Postmortem Examination

External examination of the cadaver showed an obese, stunted body with hair over the entire abdomen and on the chest. There were many hemorrhages beneath the skin of the limbs. The pituitary fossa and its contents were preserved. There was considerable edema of the lungs with an excess of frothy fluid in the bronchi. The left ventricle was hypertrophied and dilated, and the heart muscle pale and mottled. Extensive arteriosclerosis was found throughout the vessels. The liver was passively congested and the pancreas greatly reduced in size. The spleen and suprarenals were normal. The kidneys, which were rather small, showed scarring of the surface, which had a "flea-bitten" appearance. There were several hemorrhages beneath the mucosa of the bladder. The uterus was infantile in type while the ovaries were small and without visible evidence of Graafian follicles.

Again to review this report, focusing our attention once more on the cardiovascular renal problem, we have marked hypertensive hypertrophy of the left ventricle, diffuse arteriosclerosis, a granular kidney, with hemorrhages into the kidney, bladder and skin, and finally terminal pulmonary edema, which is so commonly seen in death associated with malignant nephrosclerosis.

Microscopic Examination

The entire kidney is severely injured by a chronic diffuse pathological process involving the vessels, the glomeruli, the tubules and stroma, leading to an almost complete reconstruction of the parenchyma and sclerosis of the interstitial tissue. The vessels show a varied picture. In a branch of the *renal artery* the intima is thickened by a narrow polster made up of a fibrillary ground substance staining blue by the Mallory anilin blue stain and showing flecks, shreds, and clumps of fibrin near the endothelial surface. The most recently formed portion of this polster is adjacent to the endothelium where it appears to have an almost semifluid appearance in which fibrils are poorly formed and appear more like lines and threads of coagulated protein, which show no definite order or arrangement. Farther away from the lumen this polster varies in structure and takes on rather a band-like arrangement in which cells are separated by well formed collagen fibrils. Just inside the original elastic interna there is seen in the Mallory anilin blue stain a bluish yellow, clear hyaline band, very narrow and showing fine reddish dots. This lamella in the elastic tissue-stained preparation is moderately positive so that we have here probably the beginning of a second elastic lamella arising in ground substance. The original elastica interna is fragmented, stains irregularly and in places is impregnated with calcium. The media of this vessel shows two interesting features: first, a hypertrophy of the individual muscle fibers, and second, a great increase in fibrillary ground substance between the muscle fibers. The adventitia is little changed. This vessel is large, the wall is generally thickened and the lumen is larger than normal. As one follows the large artery to the *interlobar* branches one finds in the latter a similar change, namely, a slight intimal and medial thickening, and the lumen is wider than that of a normal interlobar artery in a patient of the same age. In places the smooth muscle fibers show regressive changes, with disintegration and disappear-

ance. In the *arcuate arteries* one again sees this vascular hypertrophy, with dilatation of the lumen and regressive changes in an already hypertrophied muscular media. The *lobular arteries* show a striking change from the three sizes of vessels already described (the renal, interlobar and arcuate). Here the media is devoid of muscle fibers. It is represented merely by a blue-staining fibrous wall that in some places is not clearly defined from the surrounding stroma. Here and there an occasional muscle fiber is still recognizable. The basement membrane is not swollen and, except for areas where it has disappeared or ruptured, appears unchanged. Between the endothelium and the basement membrane one finds a bluish fibrillary substance (using the Mallory anilin blue stain) often containing delicate fibrin threads and not infrequently filled with large coarse clumps of fibrin and red blood cells. Occasionally fibrin and red blood cells may be traced throughout the wall. In these vessels the lumina are greatly narrowed and not infrequently obliterated by fibrin thrombi, or simply by the accumulation of subendothelial ground substance or the accumulation of nests of large "foam cells." There is no lamellar elastosis in these lobular arteries. The *afferent arterioles* to the glomeruli show changes resembling those in the lobular vessels just described, with necrosis of the wall and fibrin thrombi in the lumen on the one hand and old healed sclerotic vessels with occluded lumina on the other. The type of sclerosis here is characterized by a lamellar arrangement of fibrous tissue beneath the endothelium in which cells and fibrils form concentric whirls within one another, greatly narrowing the lumen.

The *glomeruli* show a varied picture. About 70 per cent of those seen in the sections examined show a rather characteristic ischemia, together with an increase in cells and ground substance, and in contrast to the normal glomerulus they are large. There are clusters of glomeruli usually just beneath the capsule which show the simple hyaline transformation with thickening of the capsule. The most interesting glomerular lesion is the fresh fulminating degenerative and inflammatory lesion associated with aneurysmal dilatation of the capillaries, hemorrhage and fibrin within the lumina and throughout the ground substance, so characteristic of malignant nephrosclerosis. Where this lesion is somewhat older there is proliferation of both endothelium and epithelial cells with desquamation and adhesions between the capillary loops and from the

capillary loops to the capsular wall. This may be associated with proliferation of the cells along the capsular wall. Where the lesion has healed many of the cells have disappeared and the glomerulus itself may no longer be easily recognizable. One finds acute lesions, others that are healing, others that have healed, and still others showing recurrent fresh lesions in glomeruli that have long ago healed. These lesions appear at times isolated and at times in small clusters of glomeruli fed by the same lobular artery.

The *tubules* show as variable a histological picture as that in the arteries and glomeruli, and like the changes in both of those one finds both fresh and old lesions. The most interesting change is the almost complete absence of well differentiated proximal convoluted tubules. A search for such clearly recognizable proximal convoluted tubules in which one seeks a rather characteristic type of epithelium reveals only here and there small scattered islands; and the cells lining these show albuminous granular degeneration, hyaline droplet degeneration and, in places, necrosis. The great majority of tubules are small, collapsed and poorly differentiated, and lined by small atrophic cuboidal cells. The lumina are narrow and contain little precipitated protein. A third type of tubule commonly found also lacks differentiation and is characterized by marked dilatation with endothelial-like cells lining the wall. Mitoses in such tubules are quite common and their lumina contain precipitated albumin, a few polymorphonuclear leukocytes and red blood cells. In a few areas the tubules have entirely disappeared. This, however, is a rare finding and is best seen at the tips of the papillae.

The *stroma* in both cortex and medulla is increased. When stained by the Mallory anilin blue stain it appears as a blue-staining, finely fibrillar ground substance, which is most marked in areas where the tubules are small and atrophic or where they have disappeared entirely, but is also present, though to a much less degree, about the tubules that form the islands of still recognizable proximal convoluted tubules. This material has the same structure, stain and character as the material beneath the endothelium of the arteries. There are small foci of the lymphocytes limited largely to the areas of sclerosis.

The *veins and capillaries* are dilated, especially the capillaries surrounding the tubules, but there are no hemorrhages from these vessels into the stroma.

The *basement membrane* in vessels, glomeruli and tubules shows a series of interesting changes. In the arterioles, as already mentioned, it is in places broken up and absent. In the glomerular capillaries it is separated from the endothelium by a newly formed, finely fibrillar ground substance. The basement membrane forming the glomerular capsules is here and there thickened. On the tubules, especially where they have collapsed, the basement membrane is thickened and somewhat irregular, and here and there one finds a fine fibrillary ground substance between the collapsed epithelium and the original basement membrane — a picture corresponding very closely to the accumulation of fibrillary ground substance beneath the endothelium in the smaller vessels.

To summarize these histological changes, we find a severely damaged kidney; the larger arteries show vascular hypertrophy, the smaller show regressive changes, necrosis, thrombosis and hemorrhage. Some show healed lesions, others fresh lesions and still others show chronic lesions, occasionally with fresh hemorrhage superimposed. The glomeruli for the most part are still readily recognizable, being large, anemic and rich in cells. There are areas in which the glomeruli show acute, healing, healed, chronic and recurrent degenerative and inflammatory processes characterized by hemorrhage, necrosis and cellular proliferation leading in places to half-moon formation within the capsule. The tubules are severely injured; only nests of recognizable proximal convoluted tubules are present. The majority are small and atrophic while others are dilated, poorly differentiated and filled with coagulated protein, desquamated epithelial cells, polymorphonuclear leukocytes and erythrocytes. The stroma is diffusely increased, the veins and capillaries are congested. Such findings as these are neither compatible with chronic glomerulonephritis nor with benign nephrosclerosis, but are characteristic of chronic malignant nephrosclerosis which has been progressing with remissions for several years.

In keeping with this picture of malignant nephrosclerosis the spleen, liver, intestine and ovary reveal the same variation and character of histological changes in the smaller blood vessels. In the spleen many of the small arterioles show fibrin throughout the wall and occlusion of the lumen, and still others show marked swelling of the basement membrane with partial occlusion of the lumen. In the ovary these changes are especially marked where

some of the small vessels are almost completely transformed into walls of fibrin.

CASE 2. H. P. (case reported by Cushing [3]), an unmarried, white female, 33 years of age, entered the hospital with a history of two periods of amenorrhea. The first attack was of 1 year and 8 months duration, occurring when the patient was 20 years of age. The present attack began 1 year and 3 months ago. She was born of healthy parents and attained normal adolescence at the age of 13, when she later grew into an intelligent, vigorous and ambitious young woman. She entered college at 18 but became unhappy there and withdrew at the end of the second year. She ascribes this to restlessness and emotional instability. In 1919, when she first ceased to menstruate, she developed a ravenous appetite, gained weight rapidly, particularly in the face and abdomen. During the summer of 1919 she broke her ankle. Purplish striae of the body and arms began to appear at that time. In December of 1919, she found herself easily fatigued and acquired a definite polyuria and polydipsia. At the same time headaches occurred, with blurred vision, tinnitus, dizziness and numbness of the hands.

Toward the end of February 1920, because of a sudden fainting attack, she came under the care of Dr. E. P. Joslin, who found there was a moderate hyperglycemia with glycosuria, and a basal metabolic rate of −30 per cent. On March 13, 1920, she was first seen briefly in consultation with Dr. Joslin. The facial hypertrichosis and the peculiar disposition of the adiposity with extraordinarily widespread striae atrophicae, associated with a moderate hypertension of 140/100, indicated a polyglandular syndrome. By January 1921, she had become increasingly hirsute and "bloated" in appearance. At this time she entered the Neurological Institute in New York where she was given baths, exercises and glandular preparations. After 4 weeks the weight was reduced, the hirsuties had disappeared and normal menstruation was resumed. From this time, for a period of 5 years, she continued under various combinations of glandular treatment and regarded herself as reasonably well.

In 1926 the face again began to get heavily bearded, necessitating the use of a razor. A year later tonsils, adenoids and impacted wisdom teeth were removed and it was noticed that the blood pressure was high, 155/115. In 1929 she had a "nervous breakdown," and the following year, while being studied at the Evans Memorial Hospital, it was noticed that the urine showed some albumin and an occasional hyaline cast with a normal phthalein test. She had a low sugar tolerance, a fluctuating hypertension, a basal metabolic rate of −14 per cent and cardiac enlargement. In January 1931 the menstrual periods, after having been essentially regular for 10 years, ceased, and in July she was found to have a marked hypertension varying from 220 to 250 systolic.

In 1932 she fell and fractured the humerus. The following summer polydipsia, occipital headaches, palpitation, shortness of breath and swelling of the feet and ankles were present. The fatness of the face and shoulders, dryness and pigmentation of the skin, cyanosis of the dependent hands and feet had markedly increased. It was observed that large ecchymoses would follow the slightest bruise and that a cut or scratch would bleed excessively. At this juncture, in October 1932, she was referred to the Peter Bent Brigham Hospital for study.

The patient was a rather tall woman, 5 feet, 9½ inches, weighing 63.5 Kg., with a peculiar moon-shaped, recently shaven face with clipped eyebrows. The eyes were puffy and there were posterior cervical and supraclavicular fat pads. She was not appreciably round shouldered and though not particularly abdominous the parietes were somewhat pendulous and flabby. The extremities did not participate in this adiposity. Over the arms, axilla, breasts, abdomen, hips, groins and thighs were an extraordinary number of broad, pale striae atrophicae. The lower extremities showed marked pigmentation and scarring of the dry and scaly skin, with several large and fading ecchymoses from recent trivial contusions.

The blood pressure averaged 220/170, the urine showed a trace of sugar and of albumin with no renal elements. There was a variable polyuria amounting to about 3 liters. The basal metabolic rate was −10 per cent. The detailed blood examination showed 4,720,000 erythrocytes, with a hemoglobin (Sahli) of 106 per cent. The non-protein nitrogen was 46.97 mg. and the cholesterol 192.3 mg. per cent. Roentgenograms showed slight diffuse atrophy of the vertebral bodies without collapse or deformity, normal detail of the cranial bones, a sella tursica of normal dimensions but hazy outline, and multiple small tiny shadows in both flanks suggesting renal calculi — a common finding in hyperparathyroidism. The patient was transferred to the Huntington Hospital where, through the kindness of Dr. Aub, her elimination was thoroughly studied. He reported essentially normal blood content for calcium phosphorus and phosphotase and normal elimination of both calcium and phosphorus. She showed a low sugar tolerance and a high nitrogen output, as shown by an average loss of 6.7 gm. daily on a balanced diet containing 56 gm. of protein.

On readmission the pituitary body was irradiated on 4 successive days without any immediate effects. She was discharged Nov. 12, 1932, and returned home and resumed her usual activities. On December 3 she retired about midnight, waking about an hour later with dyspnea and increasing cyanosis. She died 12 hours later from what was supposed to be acute pulmonary edema.

When we study this patient in retrospect from a cardiovascular renal standpoint we find that in 1919, when the patient was 20 years old, she began having headaches, blurred vision, tinnitus, dizziness and numbness of the hands. Several months later we find the blood pressure slightly elevated, 140/100. Six years later the blood pressure was again examined and had reached 155/115, and the urine contained albumin and casts. The heart at this time was enlarged. Five years later we find the hypertension had increased to nearly 250 systolic and a year later there were occipital headaches, palpitation, shortness of breath and swelling of the feet and ankles, and the patient observed that large ecchymoses would follow the slightest bruise. The physical examination at the time of hospital entry in 1932 substantiated this high blood pressure with some albumin in the urine. There was a variable polyuria and the non-protein nitrogen and cholesterol values were increased and the

nitrogen output showed an average loss of 6.7 gm. daily on a balanced diet of 56 gm. of protein. Finally, death came suddenly, probably to be explained on the basis of pulmonary edema.

From such a summary our attention is primarily focused upon the cardiovascular problem, with its hypertension, enlarged heart, headaches and disturbances in vision. The disturbances in renal function with albumin, elevated non-protein nitrogen and cholesterol stand rather in the background and yet the sequence of events must be carefully considered, and a clinical diagnosis of benign nephrosclerosis with beginning renal decompensation or malignant nephrosclerosis must be considered. The tendency to bleed when slightly bruised is found more commonly associated with the latter diagnosis.

Postmortem Examination

An autopsy was performed by Drs. Schulz, Hass and Cushing, and only the cardiovascular renal changes will be mentioned here. The heart was enlarged (695 gm.) and the large arteries, including the aorta, showed an advanced degree of atherosclerosis. The kidneys had slightly adherent capsules and on section minute calculi were visible in the calices.

Microscopic Examination

The kidney is finely granular, the capsule thickened, and small hemorrhages are seen over the surface. The kidney as a whole shows only moderately severe changes, the most important of which appear in the cortex and are somewhat irregularly distributed. There is a destruction of kidney tissue and a new formation of tubules with a general reconstruction of the normal architecture. The arteries show a variety of interesting changes. The large *interlobar* arteries show marked medial hypertrophy with large, well preserved muscle fibers. The intercellular ground substance is not remarkably increased. The internal elastic lamina is intact and stains well. The intima is only slightly thickened, showing a lamellar connective tissue thickening. Practically the same changes are found in the *arcuate* and larger lobular arteries; that is, we have in these vessels a form of vascular hypertrophy with an enlargement of the vessel, a thickening of the vessel wall and a lumen larger than that of a normal person of the corresponding age. In the smaller *lobular*

arteries one sees a gradual disappearance of the muscle fibers from the largest to the smallest arterioles. The vessels are abnormally large and the media shows replacement by connective tissue. The internal elastic lamina is still preserved, and the basement membrane of the arterioles is here and there remarkably swollen, irregular, and when stained with the Mallory anilin blue stain appears reddish yellow. In other arterioles the basement membrane is unchanged and between the endothelium and this basement membrane there is an accumulation of watery-like material forming in places a very fine network which, when stained with the Mallory anilin blue stain, appears distinctly blue. This accumulation beneath the endothelium leads in places to almost complete obliteration of the lumen. In other vessels of similar caliber, where the process is older, one sees a lamellated arrangement of cells and connective tissue in which spindle-shaped cells appear drawn out and separated by narrow bundles of collagen, giving a characteristic "onion-like" picture. A still more striking change, though somewhat rarely seen, is the presence of fibrin and red blood cells within the wall, which at times is completely necrotic. Nests of fatty endothelial cells beneath the endothelium and fibrin in the lumen may complicate the picture. Some of the arterioles to the glomeruli are scarcely recognizable. The lumina of the vessels comprising the smaller branches of the vascular tree are greatly reduced. It is not infrequent to find extensive vascular changes without a corresponding change occurring in the capillary loops of the glomeruli.

The *glomeruli* are relatively little changed. By count, between 90 and 95 per cent are still preserved, the most of which, however, are large, ischemic and show an increase in cells and intercellular substance. Here and there, usually occurring in small groups of two and three, glomeruli show simple hyaline transformation with connective tissue thickening of the collapsed capsule. Here and there, even in areas showing rather advanced atrophy of the tubules, one finds well preserved glomeruli, rich in blood, showing neither cellular nor intercellular changes. The most striking change is that so characteristic of malignant nephrosclerosis, namely, the capillary dilatation, hemorrhage into the capsular space and fibrin occluding the capillary lumina and extending out into the somewhat loose intercellular basement membrane and ground substance. Regressive changes in the epithelial cells are occasionally found associated with

proliferation and desquamation. Glomeruli showing these changes are infrequent. They appear singly and in small groups. Somewhat older changes, including the chronic and the healed lesions with simplification of the dilated capillaries, adhesions of the capillary loops with each other, and with the capsular wall, and even half-moon formations are also found, but only rarely.

The *tubules* show the usual varied picture, such as is seen in malignant nephrosclerosis. Unlike the former case, however, many of the proximal convoluted tubules are still moderately well preserved, showing the characteristic type of epithelium. They are moderately hypertrophied and in places show regressive changes leading at times even to necrosis. Most of the tubules are changed. They are small, atrophic, collapsed and bordered with small cuboidal, undifferentiated epithelial cells often containing in the lumina small hyaline casts. Here and there and usually occurring in islands are nests of tubules showing dilatation; such tubules are bordered with elongated endothelial-like cells showing frequent mitoses, and frequently contain remnants of necrotic desquamated cells, precipitated albumin and polymorphonuclear leukocytes. Stains for bacteria are negative. The necrotic cells are incrustated with salts often rich in iron and in places surrounded by large multinucleated foreign body giant cells. It is interesting that even in this group of reformed tubules hyaline droplet degeneration is already present in the cells. In the medulla where casts incrustated with iron have remained fixed the surrounding epithelium has totally disappeared, leaving this foreign body surrounded by connective tissue.

The *veins* throughout the kidney and the *intertubular capillaries* are unusually dilated and one sees very well the very close relation that the intertubular capillaries bear to the tubules, being separated only by a basement membrane, an anatomical relation similar to that of the capillary tufts of the glomerulus. Occasionally one sees petechial hemorrhages into the stroma of both cortex and medulla from these dilated capillaries.

The *basement membrane* of the arterioles is markedly swollen and rich in lipoids but this kidney, like the former, shows no double refractile fat. Along the capillary loops of the glomerular tufts the basement membrane seems loose, lax, and increased and fibrillated, but not swollen like that of the arterioles. The basement

membrane forming the capsule in places is rather coarse but quite uniform and shows no papillary bulging. Along the tubules, and especially those that are collapsed, it is swollen and irregular, and here and there fluid-like material slightly fibrillar has collected between the collapsed epithelium and the basement membrane.

The *interstitial tissue* is unevenly increased and shows foci of lymphocytes, especially in areas of tubular atrophy and disappearance. In the medulla the stroma is diffusely increased, yet there is practically no total disappearance of tubules.

To summarize these histological changes, we find a moderately damaged kidney with changes involving the blood vessels, glomeruli, tubules and stroma. The lesions for the most part are chronic and fairly advanced. From the character and extent of the histological lesion alone there is no basis for one to believe that either through the vascular, glomerular, or tubular changes this patient should suffer from severe renal insufficiency. The lesions, being largely of a chronic nature, warrant the diagnosis of a slowly progressive type of malignant nephrosclerosis. This is substantiated by the finding of similar, if not more severe, lesions of the same character in many of the other organs of the body.

DISCUSSION

A study of the etiology of malignant nephrosclerosis has, since the work of Volhard and Fahr, occupied the attention of many investigators. A recent paper by Schürmann and MacMahon [7] reviews the work that has already been done up to the present time. Without question it would appear that the anterior lobe of the pituitary, and especially the secretion of the basophilic cells, may, in some cases, play a very important rôle in the etiology of this important cardiovascular renal syndrome. Most cases of malignant nephrosclerosis, however, are not characterized by such signs of pituitary basophilism as adiposity, disturbances in secondary sexual characteristics and osteoporosis.

For years clinicians and pathologists considered the bony changes of osteitis fibrosa deformans and osteodystrophia fibrosa cystica as being one and the same fundamental disease. Only with the discovery of the important rôle played by the parathyroid in cystic disease of bone were the clinical and histological differences in these two diseases accepted as definite and distinct entities. Now the

question may be asked — can we distinguish a particular group of cases of malignant nephrosclerosis clinically or histologically which specifically belong to the syndrome of basophil tumors of the anterior lobe of the pituitary? From a study of fifty cases of malignant nephrosclerosis one finds variations in the clinical and pathological picture, but clinically these cases of malignant nephrosclerosis associated with typical signs of pituitary basophilism, as described by Cushing, belong to a very definite and distinct group. Comparing the vascular changes in these two cases of basophilic adenoma of the anterior lobe of the pituitary with the vascular changes in many other cases of malignant nephrosclerosis which did not show pituitary basophilism, we could find no single change or group of changes that would permit us to distinguish one case specifically from another.

Summary

Two cases of basophilic adenoma of the anterior lobe of the pituitary, one reported by Bishop and Close and the other by Cushing, have been discussed again from a cardiovascular renal standpoint in which it is shown that the cardiovascular renal lesion present in these two cases corresponds to the picture originally described as malignant nephrosclerosis by Fahr.

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STUDIES ON INFLAMMATION

X. THE CYTOLOGICAL PICTURE OF AN INFLAMMATORY EXUDATE IN RELATION TO ITS HYDROGEN ION CONCENTRATION *

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For many years it has been known that the cytological sequence in acute inflammation is characterized in the earliest stages by an active emigration of polymorphonuclear leucocytes. After a time this is followed by an infiltration of mononuclear phagocytes. The latter have been designated by various names, the most satisfactory of which is perhaps that of "macrophage," originally suggested by Metchnikoff. In acute inflammation the polymorphonuclear cells that leave the circulating blood stream form the chief cellular constituents of the early exudate. The mononuclear phagocytes or macrophages increase in number in the later stages. These cells act as scavengers when the inflammatory irritant has been overcome. They engage actively in engulfing and digesting polymorphonuclear leucocytes, red cells, and various necrotic materials resulting from the acute inflammation. The orderly cytological sequence in the development of an inflammatory reaction was first pointed out by Borrel [1] and then by Durham [2] about forty years ago. The subsequent studies of Beattie [3] extended considerably the original observations of Durham. This sequence is true of the majority of inflammatory reactions caused either by bacteria or by chemical irritants. It is noteworthy that during the first twenty-four hours after their inoculation into normal tissues both tubercle and typhoid bacilli produce the same type of cellular changes as do various forms of pyogenic bacteria such as *Staphylococcus aureus*.[1, 4, 5] The difference in the leucocytic response found with various types of inflammatory irritants seems therefore to be one of degree rather than of kind.

No adequate explanation has been offered for this fundamental process. A number of years ago various investigators, particularly

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Opie, studied the action of intracellular proteolytic enzymes from leucocytes of an inflammatory exudate.[5, 6] Müller,[7] and subsequently Opie, showed that polymorphonuclear leucocytes contain an intracellular enzyme that acts in a slightly alkaline or neutral medium, but is almost wholly inactive in an acid reaction (0.2 per cent acetic acid). Opie designated this intracellular enzyme "leucoprotease." The action of this polymorphonuclear enzyme occurs only within the leucocyte, for in the plasma of an inflammatory exudate its activity is inhibited owing to the action of anti-enzymes. The earlier observations of Opie on the presence of antiferments inhibiting the action of leucoprotease have been recently confirmed by Weiss.[8] Opie furthermore demonstrated that the mononuclear phagocytes that accumulate in the later stages of the inflammatory reaction contain an enzyme causing active digestion of protein in a weakly acid medium, but almost entirely inactive at a neutral or alkaline reaction. The enzyme of the mononuclear phagocyte has been called "lymphoprotease."

It is conceivable that particles in an inflammatory exudate prior to being phagocytosed by a given type of leucocyte may tend to have on their surfaces a hydrogen ion concentration approximating that of the intracellular proteolytic enzyme capable of digesting them. If this assumption is correct then it is to be expected that the inflammatory exudate in which such particles are immersed would gradually increase in its acidity concomitantly with the shift from polymorphonuclear to mononuclear phagocytes. The question arises therefore as to whether or not there is a correlation between the pH of the medium and the cytological picture during the development of an acute inflammatory reaction. In this connection it is to be noted that Opie recorded several measurements on the reaction of a pleural exudate.[9] At no time during a 5 day period of the inflammatory process did the alkalinity of the exuded serum disappear; it was, however, less than that of the blood serum. It may be mentioned also that as the inflammation progressed there seemed to be a slight decrease in alkalinity. Since, however, no precautions apparently were taken to avoid loss of carbon dioxide during withdrawal and testing of the exudate, the validity of these measurements as absolute figures may be open to some question. Lord [10] in his studies on proteolytic enzymes in the pneumonic lung concluded that during the course of the disease a gradual increase

in the hydrogen ion concentration of the exudate probably occurs. He conceived resolution to be the result of this increased hydrogen ion concentration, which eventually activated a proteolytic enzyme having a range of optimum reactivity at a pH of 6.3 and 5.2.

Rous found that death of small cell aggregates resulted in the development of an alkalinity of these cells, owing to seepage into them of alkaline body fluids.[11] He recognized that the chemical changes that take place in small necroses differ in important respects from those occurring in large masses of dead tissue. He was led by his observations to conclude that very pronounced inflammatory edemas yield alkaline fluids but that "inflammation, as such, conduces to local acidosis."

None of the studies mentioned has correlated the pH of the inflammatory exudate with its differential leucocyte count. The object of the present communication is to report data on the trend of the hydrogen ion concentration and to relate this to the cellular changes in an exudate obtained at various intervals from an acute inflammatory area. The relation obtained suggests that the prevailing hydrogen ion concentration may be an important factor in determining at a given time the cytological picture of an inflammatory exudate.

Experimental

Method: Pleural exudation was induced by the injection under ether anesthesia of 1.5 to 2 cc. of turpentine into the right chest of dogs.[9] Several hours to 1 day following the injection of the irritant a sample of the exudate was withdrawn by means of a Luer syringe with a hypodermic needle. The latter was of large caliber and filed off at the end, in order to diminish the chance of injury to the lungs. To prevent coagulation several glass beads were placed in the barrel of the syringe. When in one experiment about 0.5 cc. of 0.1 per cent heparin in Tyrode solution was employed as an anticoagulant, essentially the same readings were obtained as with the use of glass beads. Upon withdrawing the sample of exudate the syringe was shaken quickly for a few seconds and several smears were made on coverslips and slides. The remaining part of the exudate was immediately transferred under paraffin oil into a test tube.

Measurements of the pH were always performed within a short interval after withdrawing the sample of pleural exudate. The bicolor system of standards, as described by Hastings and Sendroy,[12] was employed in determining the hydrogen ion concentration. These investigators had found close agreement when results obtained by this method were checked up with parallel electrometric pH measurements. They also had determined that the "salt and protein errors" were negligible. The standards prepared with phenol red as indicator covered a range of pH 6.7 to pH 8. In a few instances the pH of the exudate was found slightly below 6.7. The reading in such cases was obtained

TABLE I

The Hydrogen Ion Concentration and the Cytological Picture in Acute Inflammation

Dog No.	Interval between injection of irritant and removal of exudate	Differential leucocyte count of inflammatory exudate			pH of inflammatory exudate	Differential leucocyte count and pH of blood			
		Polymorphonuclears	Lymphocytes	Mononuclear phagocytes		pH	Polymorphonuclears	Lymphocytes	Mononuclears
	hrs. : mins.	*per cent*	*per cent*	*per cent*			*per cent*	*per cent*	*per cent*
4	19:15	78.0	2.0	20.0	7.45				
	43:45	78.0	1.0	21.0	7.23				
	67:15	87.0	1.0	12.0	7.23				
	93:08	31.0	3.0	66.0	6.97				
	115:00	26.0	2.0	72.0	6.95	7.07	83.0	7.0	10.0
5	22:57	79.0	3.0	18.0	7.15				
	47:00	9.5	0.5	90.0	6.8				
	71:15	2.0	0.0	98.0	6.6	7.4	77.0	13.0	10.0
7	24:10	68.5	7.0	24.5	7.4				
	48:30	72.6	3.4	24.0	7.35				
	72:45	75.7	6.0	18.3	7.45				
8	24:50	90.0	0.5	9.5	7.23				
	47:35	87.0	2.7	10.3	7.13				
	71:45	63.25	0.5	36.25	6.78				
	100:05	59.0	1.0	40.0	6.98	7.1			
3	23:00	90.0	1.5	8.5	7.05				
	48:10	53.5	1.0	45.5	6.75				
	72:40	7.0	1.0	92.0	6.6				
	95:40	10.0	3.0	87.0	6.65	7.23	71.0	7.5	21.5
2	23:42	69.3	8.3	22.3	7.4				
	47:37	74.3	2.6	23.0	7.4				
	71:12	83.0	2.0	16.6	7.25				
	95:22	88.3	0.6	11.0	7.25				
2-A*	19:35	..	..	..	7.23				
	43:35	13.0	3.0	84.0	6.6				
	67:10	3.5	0.5	96.0	6.7	7.23	77.0	10.0	13.0
10	6:15†	..	..	..	7.55				
	24:25	40.5	12.5	47.0	6.93				
	48:15	63.5	11.5	25.0	7.0				
	72:38	74.0	6.0	20.0	7.08				
	96:15	74.0	2.0	24.0	7.28				
	120:15	76.0	0.0	24.0	7.28				
	145:35	74.0	3.0	23.0	7.50				
	169:35	78.5	7.5	14.0	7.55				
11	24:15	26.0	9.75	64.25	6.98	7.28	69.0	18.0	13.0
9	52:55	72.0	6.0	22.0	7.05	7.05			
16	18:00	71.0	10.0	19.0	7.55	7.28	83.0	4.0	13.0
12‡	23:30	81.0	1.5	17.5	7.25				
	48:35	52.0	1.0	47.0	6.95				
14‡	23:25	90.0	0.66	9.33	7.2				
	47:20	64.0	4.5	31.5	6.95				
	75:20§	87.0	0.0	13.0	7.28				
13¶	24:06	87.5	2.0	10.5	7.4				
	49:02	77.5	2.5	20.0	7.5				
	71:45	74.0	6.0	20.0	6.98				
	95:30	10.0	8.5	81.5	6.8	7.45	76.0	9.0	15.0
15¶	23:25	86.0	1.0	13.0	7.35				
	47:15	42.5	2.0	55.5	6.8				
	75:40	17.5	3.5	80.0	6.75				

* Dog 2-A is Dog 2 reinjected with turpentine in the right pleural cavity 35 days after the first injection with the irritant.

† Upon removal of the exudate 6 hours and 15 minutes after the injection of the irritant practically no leucocytes were found.

‡ A total of 8 to 9 cc. of a phosphate buffer mixture at pH 6.78 was injected in divided doses, subsequent to the irritant, into the right pleural cavity.

§ This particular sample of the exudate was removed immediately after killing the animal.

¶ A total of 15 to 20 cc. of a phosphate buffer mixture at pH 7.28 was injected in divided doses, subsequent to the irritant, into the right pleural cavity.

by the use of bromcresol purple as indicator and represents only a first approximation. The thorough studies of Drury and Rous had shown that in the animal body, at least, the observed colors in tissues vitally stained with phenol red or bromcresol purple cannot be ascribed to indicator errors resulting from association of the phthalein with tissue materials.[13] For this reason it is believed that the readings obtained by adding under oil 0.2 cc. of the pleural exudate to 4 cc. of a standard phenol red indicator solution (made up in saline and adjusted to about pH 7.4) are reasonably reliable and do not represent indicator errors. In several instances, in spite of an appropriate saline control tube, the turbidity of the exudate rendered readings somewhat difficult, so that centrifugalization for a few minutes had to be resorted to. The determinations were always made after the tubes had been immersed in a water bath at about 38° C for several minutes.

The differential leucocyte counts were made from smears on coverslips and slides. The cells were stained by the Wright method. As a rule several hundred cells were counted in each sample. In computing the percentage of polymorphonuclears and mononuclears cells were frequently encountered that were so degenerated as to render their identification difficult. These were not included in the final counts.

Samples of the pleural exudate were withdrawn daily and studied as described for a period not exceeding 1 week after the injection of the irritant. At the completion of an experiment the animal was anesthetized under ether and frequently a sample of blood was withdrawn from the femoral vein in a syringe containing about 0.5 to 1 cc. of 0.1 per cent heparin in Tyrode solution. The pH of the blood and its differential leucocyte count were determined. The administration of ether was continued until the death of the animal. A post-mortem examination was performed and specimens of the inflamed pleura and right lung were placed in 10 per cent formaldehyde for subsequent histological examination.

Results

The results of all the 15 experiments performed are summarized in Table I. A cursory examination of the data shows that in 8 out of 12 animals in which the pleural exudate was studied from day to day, as the inflammatory reaction progressed, the hydrogen ion concentration changed from an alkaline to an acid pH. The change in the reaction toward a definite acidity occurs usually 2 or 3 days after the injection of the irritant. Concomitantly with this decrease in the alkalinity of the exudate there is a change in the differential leucocyte formula. The percentage of polymorphonuclears falls, whereas the percentage of mononuclear phagocytes correspondingly rises. The percentage of lymphocytes evidently plays no significant rôle in these cellular changes. Composite graphs of all experiments showing the parallelism in the fall of the pH and the drop in the percentage of polymorphonuclear leucocytes appear in Chart 1.

Since the percentage of polymorphonuclear leucocytes represents virtually the reciprocal of that of the mononuclears, the latter were not plotted on the chart. An examination of the data reveals that the percentage of polymorphonuclears predominates over that of the mononuclears whenever the pH is alkaline. A rise in the hy-

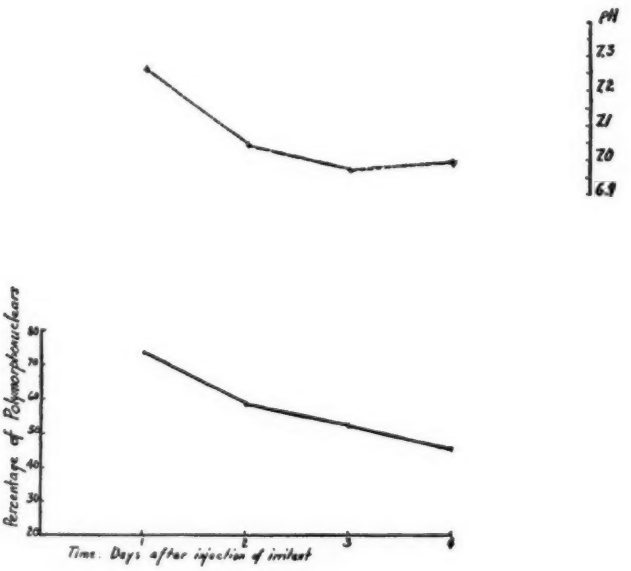

CHART 1

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural inflammatory exudates. Composite graphs of 12 experiments.

- - - - - + - - - - - pH
——— • ——— Percentage of polymorphonuclear leucocytes.

drogen ion concentration is immediately or at least very soon followed by a fall in the percentage of polymorphonuclear leucocytes. By studying the hydrogen ion concentration one can fairly well predict the cytological picture in the exudate, and *vice versa*. The correlation is evidently very close. In Dogs 7, 2, and to some extent in Dog 10 the pH failed to become acid concomitantly with the progress of the inflammatory reaction. The counts correspondingly reveal a predominance in the percentage of polymorphonuclear cells throughout the period of the experiments (Chart 2, Dog 7).

In Dog 11 the per cent of polymorphonuclears appears surprisingly low for an inflammation of only 24 hours duration; the pH here is 6.98. To summarize, the point under discussion can perhaps be illustrated by the following calculation from Table I. In 31 counts in which the percentage of polymorphonuclears ranged from 60

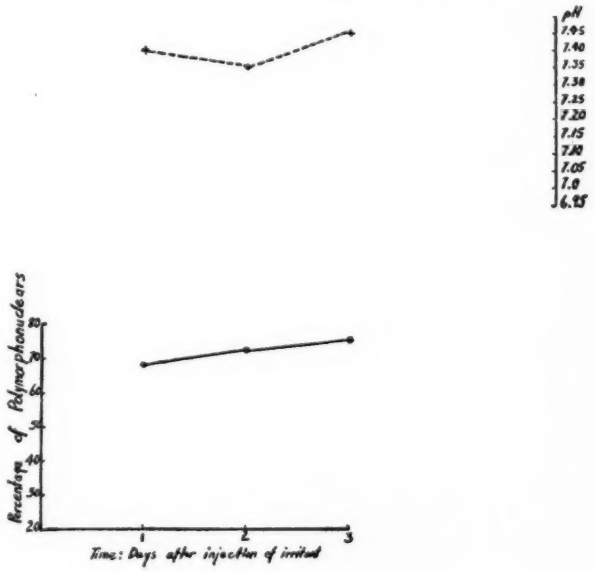

CHART 2

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural exudation from Dog 7. Note that the pH remains alkaline and that the percentage of polymorphonuclears maintains a high level throughout the duration of the experiment.

- - - - - + - - - - - pH
——— · ——— Percentage of polymorphonuclear leucocytes

to 90 per cent, the pH averaged 7.25. Contrast this with 16 counts in which the percentage of polymorphonuclears ranged from 2 to 60 per cent, and the pH averaged 6.80.

Having obtained definite evidence of the close parallelism between changes in hydrogen ion concentration and in the differential leucocyte formula in acute inflammation the question arose as to which comes first, the changes in the pH or the cellular modifica-

tions. The present data are highly suggestive in answering this question. An examination of the results obtained in the case of some individual experiments points out that the increase in the hydrogen ion concentration evidently precedes the fall in the percentage of polymorphonuclear leucocytes. Chart 3 illustrates this

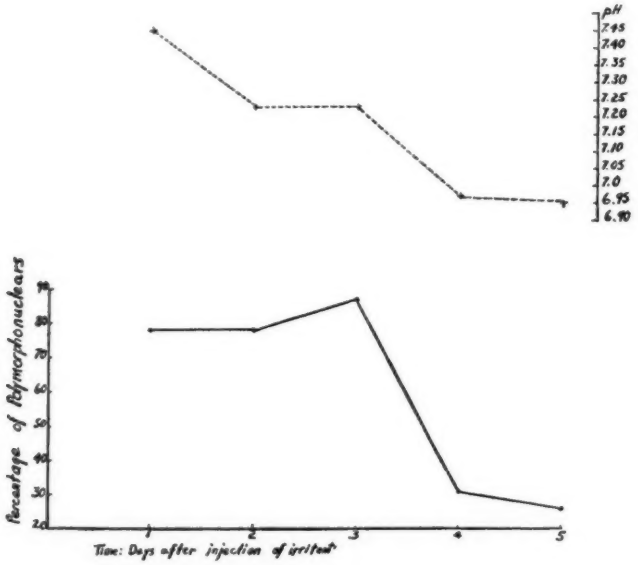

CHART 3

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural exudation from Dog 4. Note that the pH steadily declines during the first 2 days, while the percentage of polymorphonuclears remains at a high level.

– – – – – + – – – – – pH
——————•—————— Percentage of polymorphonuclear leucocytes

fact to some extent in the case of Dog 4. Whereas the pH of the exudate steadily declines from an initial value of 7.45, the percentage of polymorphonuclear leucocytes remains high. There was an abrupt fall in the percentage of polymorphonuclears only when the pH reached 6.97. The point is perhaps better exemplified in the case of Dog 13, Chart 4. For the first 2 days the pH was alkaline, 7.4 and 7.5 respectively. The percentage of polymorphonuclears was high, 87.5 and 77.5. On the 3rd day there was an abrupt fall

in pH to 6.98. The percentage of polymorphonuclears, however, was still high, namely 74. On the 4th day the pH was lower than on the preceding day, namely, 6.8. The exudate contained only 10 per cent of polymorphonuclears. Hence in this experiment the sharp rise in hydrogen ion concentration definitely preceded the fall

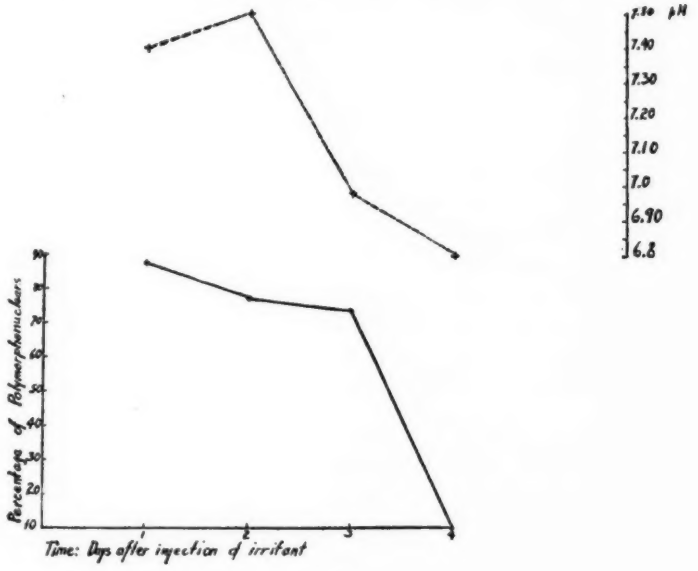

CHART 4

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural exudation from Dog 13. Note that the abrupt fall in the pH precedes the sharp drop in the percentage of polymorphonuclear leucocytes.

- - - - - + - - - - - pH
——— · ——— Percentage of polymorphonuclear leucocytes

in the percentage of polymorphonuclear leucocytes. The latter followed the decrease in alkalinity only after the lapse of a definite period. This is definite evidence that the fall in pH precedes the changes in the cytological picture. The hydrogen ion concentration may thus possibly be the regulating factor in determining the differential leucocyte formula of an exudate. In view of what is known of the mechanism of intracellular enzyme action in leucocytes, a physicochemical regulatory mechanism of this type would not be

wholly unexpected. That the fall in pH seems to precede the drop in the percentage of polymorphonuclear leucocytes is quite evident from the above analysis. At the same time it is obvious on examining the data that this relation is not always evident. This seems to depend on the rapidity of the change in reaction. If the rise in hydrogen ion concentration is rapid and sharp the corresponding fall in the percentage of polymorphonuclears may occur so rapidly as to appear to be a parallel phenomenon (see Dog 15, Table I, Chart 5). When the change in reaction proceeds very rapidly the exudate smears invariably reveal numerous degenerated, swollen, and vacuolated polymorphonuclear leucocytes containing characteristically fragmented and intensely stained nuclei. Such lethal effects accompanying an abrupt change in the reaction with increase in the acidity may be an important factor in explaining suppuration at the site of inflammation. In this connection it is perhaps also interesting to note that Rous [11] in his studies on factors that determine the reaction of skin grafts came to the conclusion that the developing acidity, in the initial stages at least, when the graft was isolated from its surroundings, was referable to the elements of the tissue proper. (Almost no cells had wandered into the grafts at this time.)

Further experiments were undertaken in an endeavor to modify experimentally the pH of an inflammatory exudate and to determine the effect of such procedure on the differential leucocyte formula. Phosphate buffers (Sörensen) were prepared at pH 6.78 and 7.28. Several cubic centimeters of each of these buffer solutions were injected immediately after the introduction of turpentine into the right chest of dogs. The phosphate buffers were reinjected at intervals of several hours. The periodic withdrawal of pleural exudates showed, however, the same tendency toward an ultimate acidosis and rise in the percentage of mononuclear phagocytes (see Dogs 12, 13, 14, 15, Table I). It became clear that the buffering mechanism of the tissues at the site of inflammation was not easily influenced by the mere introduction of phosphate buffer solutions.

Postmortem examination of the right chest of dogs injected with turpentine several days previously revealed an intense serofibrinous and at times a fibrinopurulent exudate. The pleura was greatly thickened and fibrinous adhesions extended from the visceral to the parietal layers, thus forming small pouches in the pleural cavity. These contained various amounts of exudate. In agreement with

histological studies by Opie [9] such tissues provided in general the same type of information regarding the cellular infiltration as was obtained from stained exudate smears.

It was of some interest to note that animals which throughout the experiment maintained an alkaline exudate (Dogs 7, 2, and 10)

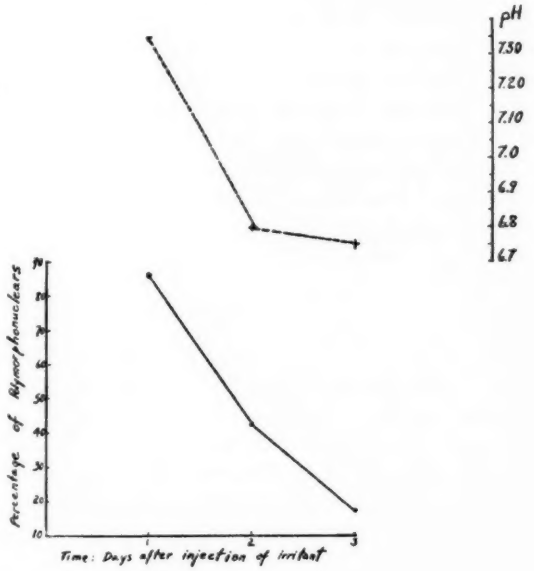

CHART 5

The hydrogen ion concentration in relation to the percentage of polymorphonuclear leucocytes in pleural exudation from Dog 15. Note the abrupt fall in the pH and in the percentage of polymorphonuclear leucocytes on the 2nd day following the injection of the irritant.

– – – – – + – – – – – pH
——————•—————— Percentage of polymorphonuclear leucocytes

appeared in much better physical condition than those whose pleural exudate gradually became acid in reaction. In the latter, dyspnea, weakness and general apathetic behavior were not infrequent.

The effect of reinjection of the same irritant was tried in the case of Dog 2. This animal received 2 cc. of turpentine intrapleurally. Its pleural exudate remained alkaline and showed a high percentage of polymorphonuclear leucocytes for 4 days. The animal was in

perfect condition at the end of the experiment. Thirty-five days later he was reinjected intrapleurally with 2 cc. of the same irritant. Within 2 days the pH was 6.6 and there was an overwhelming number of mononuclear phagocytes in the exudate (Dog 2-A, Table I). The acid pH persisted and on the 3rd day when the experiment was terminated the animal displayed some difficulty in breathing. The pH of the blood was 7.23 and contained 77 per cent of polymorphonuclear leucocytes. In a previous communication the writer [14] pointed out that an area of inflammation is ultimately walled off from the rest of the organism; the inflamed area develops its own local circulation, its own hydrogen ion concentration and its own metabolism. This view is substantiated when the data on the pH of the blood are compared with those obtained in the majority of exudates in the later stages of the inflammatory reaction (Table I).

Discussion

The results obtained in this series of experiments reveal the fact that in most instances an acute pleural inflammation induced by a strong chemical irritant such as turpentine gradually develops a local acidosis. Furthermore, the data point toward a definite relation between the hydrogen ion concentration and the cytological picture of an inflammatory exudate. The findings certainly indicate the occurrence of a parallel between the hydrogen ion concentration of the fluid medium and that favorable to the action of the enzyme of the predominating phagocyte. The relation between the two phenomena is strongly suggested by the following considerations.

In the first place, although inflammation as such conduces to local acidosis with a concomitant shift in the cell counts from polymorphonuclear to mononuclear cells, it is interesting to note that in several experiments an alkalinity was maintained throughout the period of the inflammation (see Dogs 7, 2, 10, Table I). In such cases, and only under these conditions, was there no shift in the cell counts, the percentage of polymorphonuclears remaining at a constantly high level even in the later stages of the inflammatory process. It becomes somewhat difficult to consider this state of affairs mere coincidence. Secondly, the fact that by determining the pH of the exudate the character of the cytological picture could be

fairly well predicted and *vice versa* seems to be definite evidence of some correlation between the pH and cell count. In the third place, the fact that in a few instances when the shift from alkaline to acid took place rapidly the cell change, although delayed, nevertheless invariably followed appears to warrant the inference that if there is an interdependence it is the pH that conditions the cytological picture and not the reverse order of sequence. The observations reported in this communication seem therefore to support the conclusion that the differential leucocyte picture at a given time in the development of an inflammatory reaction is a function of the pH of the exudate.

The implications of this concept are obvious. It is possible that an understanding of the histological differences of various inflammatory lesions may be facilitated through a study of their respective hydrogen ion concentrations.

Opie [5] pointed out that the studies on intracellular enzymes of leucocytes have served to explain many of the phenomena of resolution. Some of his earlier conclusions [15] on the solution of tissue with abscess deserve perhaps revision, in view of the present observations. Briefly stated, Opie's original experiments on abscess formation consisted in inducing a purulent exudation by the subcutaneous injection of turpentine. Four or 5 days later a large cavity distended with fairly thick purulent fluid was formed. The cells of this pus were separated from the serum by centrifugalization. To the cell-free pus serum, leucoprotease was added. This combination freely digested coagulated serum. On the other hand, the same polymorphonuclear enzyme in the presence of blood serum failed to digest materially the coagulated serum. From these facts Opie concluded that the anti-enzymatic action of a limited quantity of exuded serum is overcome by an increasing quantity of proteolytic enzyme set free by disintegration of polymorphonuclear leucocytes, thus accounting for the solvent effect on tissues of a purulent exudation. This conclusion is now perhaps somewhat difficult to accept; at least the data presented in this paper open the way to a different interpretation. It has been found (Table I) that as a rule 4 or 5 days following the onset of an inflammatory process induced by turpentine the resulting purulent exudate is usually characterized by an acid reaction. Opie has demonstrated that both leucoprotease and anti-enzymes are inactive in an acid medium. It is therefore

more likely that the solution of fibrin or necrotic tissue in a purulent area of inflammation is due to the activation of autolytic enzymes by the acid reaction. Enzymes of this type have been adequately described in a recent review by Bradley.[16] This interpretation appears to be more in accord with the facts since it is hardly possible to assume in view of Opie's own findings that an excess of leucoprotease from disintegrating polymorphonuclears could act in an acid medium. It seems probable that in Opie's experiment the proteolytic enzyme, leucoprotease, was inactivated by the acid cell-free pus, while at the same time there were present in this fluid autolytic tissue enzymes that possessed an optimum activity in an acid pH and were hence able to digest the coagulated serum. Opie's earlier view on the mechanism of caseation, which he considered to be the result of an accumulation of autolytic enzymes released from epithelioid cells and which acted in an approximately neutral or weakly acid medium, is not wholly dissimilar to the writer's view, as just expressed in regard to the solution of fibrin and necrotic tissues in an abscess.[17] Furthermore, it is to be noted that Opie himself pointed out in some of his later studies [9] that whereas leucoprotease may play a part early in the digestion of fibrin, the latter undergoes solution in the advanced stages of an inflammatory reaction only in the presence of weak acid. Opie [5, 9] therefore concluded that fibrin is ultimately digested by an enzyme having the character of lymphoprotease and resembling the autolytic enzymes of tissues. In an endeavor to throw further light on the question under discussion 1.5 cc. of turpentine were injected subcutaneously into the right flank of a dog. Four days later a large subcutaneous abscess containing thick viscous pus resulted. The pH of this exudative material was definitely acid in reaction, approximately 6.6. This would support the contention that an excess of leucoprotease could not possibly be the important factor in the solution of tissues in such abscesses since this enzyme is active only in an alkaline or neutral medium.

Bayliss,[18] as a result of his studies on emulsin, expressed considerable doubt as to the actual existence of true anti-enzymes as follows: "Some of the effects described as being due to them are to be accounted for by changes of hydrogen ion concentration, others to adsorption of the enzyme by a colloid." Bayliss pointed out that in his emulsin experiments the effect was found to be due merely to

diminution of the acidity of the solution. The inhibitory effect of the anti-enzyme disappeared when the solution was brought back to the initial value by the addition of acid phosphate. This idea may doubtless have considerable importance in revising our accepted concepts concerning so-called "anti-enzymes" in inflammation, especially in view of the progressive increase in hydrogen ion concentration in such pathological areas. Nevertheless, although Bayliss may be correct as far as anti-enzymes are not comparable in regard to specificity to the true antibody, still by counteracting the effectiveness of the enzyme, whether by adsorption or by changes in the hydrogen ion concentration, the anti-enzymatic effect of serum on enzymes remains a fact.

The mechanism conducing to local acidosis in inflammation is still somewhat problematical. Schade and his co-workers [19] reported that pus from acute abscesses had a pH ranging from 5.95 to 6.50; the pH of normal tissue fluids ranging from about pH 7.10 to pH 7.40. The studies of Irisawa [20] and of Ito [21] have shown that lactic acid is a constant constituent of pus. Gessler [22] has demonstrated that the oxygen consumption and the metabolic rate are increased in an inflamed area. This state of affairs would doubtless favor the development of a local acidosis unless properly compensated by an equally increased and effective fluid circulation at the site of inflammation. The writer has shown in previous studies that various foreign substances, including bacteria and electrolytes, are unable to escape readily from the site of inflammation owing to the presence of a fibrinous network and of thrombosed lymphatics. Furthermore, in acutely inflamed areas of moderately long standing a number of vascular capillaries have also been found with their lumina occluded by thrombi.[32] It is conceivable, therefore, that as acid metabolites are formed in an acutely inflamed area these tend to be fixed *in situ*, thus causing a rise in the hydrogen ion concentration of the exudate.

Summary and Conclusions

A pleural inflammatory exudate, in the majority of instances, develops a rise in its hydrogen ion concentration concomitantly with the progress of the inflammatory reaction.

When the pH of the exudate is alkaline the percentage of poly-

morphonuclears at the site of inflammation exceeds that of the mononuclear phagocytic cells.

When the pH of the exudate is approximately neutral the percentage of polymorphonuclear cells tends to approach that of the mononuclear phagocytes.

When the pH of the exudate is definitely acid large numbers of polymorphonuclear cells are found degenerated. The percentage of relatively normal appearing polymorphonuclear leucocytes is found considerably lower than that of the mononuclear phagocytes.

In some cases the pH of the exudate remains alkaline throughout the period of an acute pleural inflammation. In these instances the percentage of polymorphonuclears invariably exceeds that of the mononuclears.

By measuring the hydrogen ion concentration of an inflammatory exudate the character of the cytological picture can be predicted with a fair degree of certainty. Likewise the converse follows.

Evidence has been obtained to show that the development of a local acidosis in an area of inflammation precedes at times the changes occurring in the differential leucocyte formula of the exudate. In such cases, however, the cytological changes ultimately follow the development of the acid reaction.

The observations reported suggest that the differential leucocyte formula in an area of acute inflammation is a function of the hydrogen ion concentration of the exudate. The cytological picture in an inflamed area seems to be conditioned by the pH of the exudate surrounding the injured tissue. The present study indicates that the developing local acidosis as the inflammatory reaction progresses can adequately account for the shift in infiltration from polymorphonuclear leucocytes to mononuclear phagocytes at the site of inflammation.

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THE CULTIVATION OF MEXICAN AND EUROPEAN TYPHUS RICKETTSIAE IN THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK EMBRYO *

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In the course of studies on typhus fever continued in this laboratory efforts have been made to compare the biological and serological properties of the Mexican and the European virus strains. While the two are beyond question closely related, determinable immunological differences have recently and clearly been brought out in the vaccination and passive immunization experiments and in the serological reactions described by Zinsser and Castaneda.[1] The most troublesome difference, however, has been the fact that it has not been possible to obtain as extraordinary an accumulation of *Rickettsiae* with the European strain by the rat X-ray method as was possible with the Mexican strain, as a practical method in vaccine production. In attempting to gain more insight into the existing differences a number of experiments have been carried out in this laboratory, the chief purpose of which was to study the two varieties of *Rickettsiae* against the same biological background, other than the louse intestine in which they appear and behave entirely alike. The following experiments would not have been performed had it not been for a casual visit to this laboratory of Dr. Ernest Goodpasture, who described to us in detail his cultivation of a variety of ultramicroscopic agents by the "fertile egg" method, details of which have since appeared in a number of publications from his department.[2, 3, 4] We take this opportunity of listing him, in this manner, as a co-author. We made no changes in the technique that he described, except in point of time and temperature of incubation — matters deemed advisable in view of the experience with *Rickettsiae* gained here. The technique in brief, then, is as follows.

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METHOD

Fertile hen's eggs were incubated at 37.5° to 38° C for 8 to 9 days. At the end of this time the eggs were washed with alcohol and flamed. Windows 0.5 to 1 cm. square were cut in the shell by means of a razor blade. In most instances the inside acellular shell membrane was not injured. Hot paraffin was allowed to flow over this layer, which was then opened by cutting around the edges of the window with a pair of fine scissors. A small amount of emulsified tunica

TABLE I

Summary of Results of Cultivation of Rickettsiae in the Chorio-Allantoic Membrane of the Chick Embryo

Strain	Inoculum	Total number of eggs inoculated	No. of eggs with dead embryos	No. of eggs with living embryo	
				Positive for *Rickettsiae*	Negative for *Rickettsiae*
Mexican *Rickettsiae*	Tunica	23	13	4	6
	Spleen	10	1	1*	8
	Egg	15	9	4*	2
European *Rickettsiae*	Brain	13	9	0	4
	Spleen	12	7	2	3
	Brain and spleen	9	7	1	1

* One each by smear only.

exudate or brain and spleen material was dropped with a capillary pipette on the extra-embryonic membrane, the chorion being uppermost. A sterile coverslip was placed over the opening and it was sealed with hot paraffin. The eggs were then reincubated at 33° C and opened after 7 to 10 days for examination. Smears were stained with Castaneda's methylene blue - safranin stain,[5] and tissue fixed in Regaud's solution (potassium bichromate 2.5 gm., sodium sulphate 1 gm., water 100 cc., to which is added 20 cc. of formalin immediately before use) and later stained by Giemsa's method. In several instances the material was also inoculated into guinea pigs and a typical response was obtained in these animals. Immunity tests showed them to be protected from subsequent homologous infections. Cultures for bacteria were made from the eggs in which no obvious signs of contamination could be observed, and in only four instances was there any growth on blood agar plates.

Results

The results of these experiments are summarized in Table I. In general it was easier to infect eggs with Mexican *Rickettsiae* and these appeared greater in number. Many embryos were found dead a few days after inoculation. This was particularly true with the European *Rickettsiae*. Even discounting the eggs with dead or autolyzed embryos, in which we never found positive results, the percentage of positive findings was so low and the amount of virus obtained so scarce that it was impossible to make vaccine from them. However, the microscopic appearance of the infected chorio-allantoic membrane seems to be of sufficient additional interest and this is, therefore, briefly described.

Details of Representative Experiments

1. *Experiments with Mexican Rickettsiae:* Eggs were received October 21, 1932, and kept at 37.5° C until October 31st. On this day three eggs inoculated with emulsified tunica material from Guinea pig 365 showed typical lesions with *Rickettsiae*.

Egg 1 kept at 33° C and opened November 3rd was negative. Egg 2 kept at 38° C and opened November 4th was negative. Egg 3 kept at 33° C and opened November 8th showed a thickened membrane over the exposed area, not adherent to the shell, but there were no signs of bacterial infection. The embryo was alive. A smear showed but few *Rickettsiae*. Part of the membrane was inoculated into Guinea pig 372, which showed slight fever but typical tunica swelling. The same animal inoculated again with tunica material from a Mexican typhus guinea pig showed complete immunity. Cultures from the egg on blood agar plates were sterile. The main part of the infected membrane was fixed in Regaud's solution and sections were cut and stained with Giemsa. Figure 1 is a drawing made from a microscopic field of this membrane under a magnification of 600 diameters. It will be seen that there are *Rickettsiae* crowded in some of the cells of the ectodermal layer which, in morphology, staining reaction with Giemsa, and in their intracellular positions, are indistinguishable from the same organisms seen in the tunica cells of guinea pigs or in the intestinal cells of human lice, polyplax or fleas.

2. *Experiments with European Rickettsiae:* Eggs were received November 18th and kept at 38° C. Three eggs were inoculated on November 22nd with material from Guinea pig 377, suffering at the time from a typical attack of European typhus infection. The temperature of the guinea pig was 106° F when it was sacrificed, 9 days after intraperitoneal inoculation. There was no scrotal swelling at the time.

Egg 1 was inoculated with brain, Egg 2 with brain and spleen, and Egg 3 with spleen only. It is important to note that, as usual, no *Rickettsiae* could be found in the tissue material inoculated.

On November 29th Eggs 1 and 2 were opened. In Egg 1 we had negative results. There apparently was no change — the membranes were not thickened and smears and sections were both negative for *Rickettsiae*. Egg 2, inoculated with brain and spleen, showed slight thickening of the membrane, and on smear a few intracellular and extracellular *Rickettsiae* were found after prolonged search. This membrane was cultured on blood agar with negative results. Sections fixed in Regaud's solution and stained with Giemsa showed a fairly large number of cells containing typical *Rickettsiae*.

Egg 3 opened on December 2nd was negative. Figure 2 represents a drawing of cells containing the organisms. In morphology, in staining reaction to Giemsa and in intracellular grouping these organisms were identical with *Rickettsiae* as seen in the cells of louse intestine infected with European typhus, and appeared to be identical with the Mexican *Rickettsiae* in the other eggs, except that perhaps they were in average measurement slightly smaller.

A part of the membrane from Egg 2 was inoculated into Guinea pig 390. This animal showed a typical temperature curve, reaching 105° F and above on the 8th and 9th day, rising to 106° F on the 10th and 11th day, and on the 12th day, when the temperature was 105.5° F, the animal was killed for histological examination and for inoculation. Guinea pigs 399 and 400 were inoculated with brain and blood from this animal and later both showed typical passage strain reactions. The brains of both Guinea pigs 390 and 399 showed characteristic brain lesions in considerable profusion. These have been independently checked by a number of experienced observers. The absence of scrotal swelling, indeed, as well as the profusion of the brain lesions, characterizes it without doubt to be of the European type of infection. This is further borne out by

the immunity test. Guinea pigs of the third, fourth, and fifth generations (Nos. 414, 418 and 443) were reinoculated, after 6 weeks to 2 months, with material from European typhus guinea pigs, and all three were completely immune.

3. *Experiments with Subcultures in Eggs:* We were interested to see if the amount of *Rickettsiae* in these membranes might not be increased by repeated transfer from egg to egg. One of these experiments is as follows.

On January 19th four eggs were inoculated with tunica material from Mexican typhus Guinea pig 447, smears from which showed *Rickettsiae*. Egg 1 opened January 28th showed a living embryo with a thickened membrane. Blood agar plate cultures were sterile. Both smear and section were positive for *Rickettsiae*. The material was ground in a mortar and inoculated into four new eggs. Among these, Eggs 6 and 7 were autolyzed, the other two showing positive results. Material from one of these, Egg 8, was inoculated into another series of four eggs, with positive results in one. While there was suggestive evidence of an increase in the number of *Rickettsiae* in the eggs of the second and third generations, this did not appear to be of sufficient degree to warrant further transfer.

Histological Examination

Changes Observed 8 to 9 Days after Infection: The appearance of the normal chorio-allantoic membrane from hatching chicks has been described by Woodruff and Goodpasture.[2] It consists of a thin layer of reticulated mesothelial tissue lined by one or two celled layers of ectodermal and endodermal tissue. At this stage of development the ectodermal layer is often absent. A great change occurs when infection with *Rickettsiae* takes place. The whole membrane with all three layers is much thickened. This naturally varies with the degree of infection. In the lightly infected membranes solitary thickening of only the ectodermal lining takes place. Usually, however, the number of cellular elements in the mesothelial layer is found to be increased. In a case of average severity the ectodermal lining is about ten cells thick, covered on the outside with a layer of degenerated cells. The endodermal lining is slightly thickened. There is a great increase in the cells with a deeply stained, round nucleus in the mesothelial

layer. These often form clumps or nodules of various sizes. Most of the cellular elements in these nodules are far too degenerated to be differentiated. A large number of cells containing coarse eosinophilic granules also appear grouped with the mononuclear cells. Often in the center of these nodules small blood vessels containing red blood cells can be distinguished. Figure 3 is a photomicrograph showing one of these nodules, together with the increased cellular element in the surrounding areas. It is interesting to note that typhus infection of the chorio-allantoic membrane gives an entirely different picture from that infected with vaccinia or fowl pox. In fact, when the organisms are few the presence of these changes often encourages prolonged search and frequently leads to subsequent finding of the organisms.

Distribution of Organisms in the Infected Chorio-Allantoic Membrane: By far the majority of the *Rickettsiae* are found in the degenerated outer layer of the ectodermal lining. There they are mostly intracellular and often in large clumps. A few are also found in the tissue spaces and these often assume a much elongated form. Pinkerton [6] considers these the most actively growing *Rickettsiae*, as shown in his tissue culture experiments. When the infection is heavy a few *Rickettsiae* may be found in some of these nodules. How they reach there it is difficult to determine. In one or two sections we have observed them along the wall of a small capillary, but we have not succeeded in demonstrating them in the endothelial cells lining these vessels. One may perhaps speculate about the formation of these nodules as starting from tissue reaction to local deposit of organisms and their metabolic products, with subsequent death of these cells due to impairment of blood supply.

Discussion

While few students of typhus fever have any doubts concerning the etiological importance of *Rickettsiae*, both in the European and the Mexican infections, there still arise occasional questions regarding the significance of the organisms found by Mooser in the tunica lesions. While we believe that the work of Mooser, as well as the extensive experimental cross-indexing of the facts bearing upon this point undertaken in this laboratory, has removed all possibility of error, every additional point of evidence is of value in so important a question.

The fact that the eggs inoculated with the Mexican tunica material develop morphologically typical *Rickettsiae* and that attempts to cultivate bacteria from these eggs are unsuccessful, added to the characteristic results of inoculation of the egg material into animals, brings further evidence to the array of proof already submitted.

In regard to the inoculations of the eggs with European material the results indicate that from tissue material in which *Rickettsiae* are apparently too few to be found by smear a culture can be produced in which they are plentifully apparent and from which the disease can again be propagated. The similarity of these cultivated *Rickettsiae* to those found in the European lice adds, we think, more strength to the assumption of the etiological significance of the *Rickettsia prowazeki*.

Furthermore, it has been of exceptional interest to us that by identical methods the European and the Mexican *Rickettsiae* can be studied against the same biological background and proved to be indistinguishable in their behavior under the same cultural conditions. This is a direct demonstration of the close similarity between the two organisms and should assist materially in removing any lingering doubt as to whether Mooser's organism may have been picked up in experimental animals during inoculation passage or not. This question was raised not long ago and every point of evidence that can clarify it is of more than ordinary importance because of the extensive endeavors to produce prophylactic vaccines and potent antityphus sera with the Mexican tunica organisms.

The fact that the inoculation of guinea pigs from the European and Mexican typhus infected eggs, respectively, has produced the two characteristic types of the disease is another point of evidence that, in spite of their close similarities, the two organisms are not absolutely identical. Though possibly derived from the same original stock, adaptation in passage through rodents, fleas and polyplax may be the cause of slight biological modifications in the Mexican strain.

Our results in the egg method of cultivation so far have not given much hope that we may obtain a sufficient number of *Rickettsiae* in this way. Several egg to egg transfers have not added materially to the yield. However, the suggestive evidence of these interesting histological appearances may perhaps throw some light on the formation of the typhus nodules in man and in experimental animals.

Summary

It has been found that both Mexican and European typhus *Rickettsiae* are able to infect the chorio-allantoic membrane of the chick embryo, although the results do not lead us to hope for its practical use in the production of vaccines. The interesting histological appearance of the typhus-infected membrane and the distribution of *Rickettsiae* are briefly described. Possible significance of this finding to clarify further the relation between *Rickettsia prowazeki* and Mooser's bodies is discussed.

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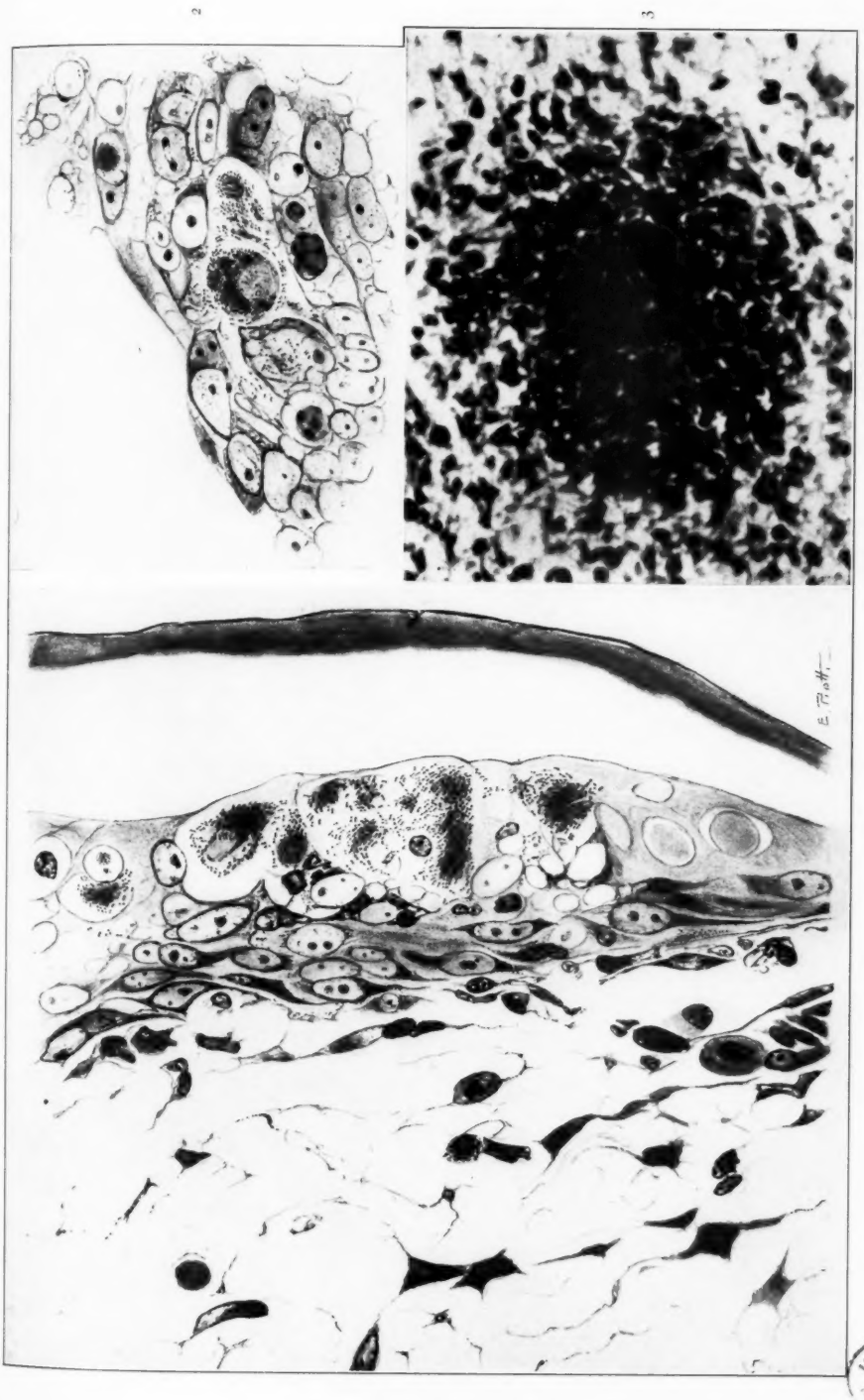
DESCRIPTION OF PLATE

Plate 70

Fig. 1. Drawing of a Giemsa-stained paraffin section of egg inoculated with tunica material from a Mexican typhus guinea pig showing *Rickettsiae* of the Mooser type.

Fig. 2. Drawing of a Giemsa-stained paraffin section of embryonic membrane from an egg inoculated with spleen and brain tissue from a guinea pig infected with European typhus of the Breinl strain, and showing *Rickettsiae prowazeki*.

Fig. 3. Photomicrograph showing one of the nodules in the mesothelial layer. × 600.



Zia

Mexican and European Typhus Rickettsiae

CYTOPATHOLOGICAL STUDIES OF MORPHINE POISONING AND CHRONIC MORPHINISM IN THE ALBINO RAT, WITH REFERENCE TO SUBSEQUENT LECITHIN TREATMENT *

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CONTENTS

I. INTRODUCTION

Recently Wen Chao Ma [1] has described in detail the cytopathology of acute and chronic morphinism in the albino rat, as well as suggested the importance of lecithin administration as a therapeutic agent during recovery from chronic morphine poisoning. Apart from reporting the pathological action of morphine upon the tissues as a whole, this author made a detailed, systematic cytologi-

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cal investigation of the behavior of the cell inclusions during temporary and prolonged treatment. These observations led him to conclude that by studying their consistent and distinctive reactions to the drug during the various phases of poisoning he was able to employ both the Golgi apparatus and the mitochondria as indicators of physiological depression under pathological conditions.

Bearing in mind the recent controversies concerning the functional significance of these cell organoids the contentions of Ma are of importance. Therefore, in view of these significant results and partly because this problem covers certain ground in which there is, apparently, some misconception, it was deemed advisable to reinvestigate this work, not only by applying more recent cytological procedures but also by including additional experiments that might lead to more conclusive results.*

II. Material and Techniques

Albino rats of both sexes, segregated shortly after weaning, belonging to the same strain and weighing approximately 195 to 215 gm., were selected for the study of acute and chronic morphinism, while animals from the same litters were isolated and kept as controls. Subcutaneous injections of morphine hydrochloride administered hypodermically were used throughout the experiments, and in order to induce acute poisoning each rodent received a single dose of 5 cc. of a 2 per cent solution (10 mg. of morphine). The rats set aside to become addicts were first given an inoculation of 3 mg. as a 1 per cent solution in water, the strength of which was increased by 1 mg. every 10 days. After a period of 7 months the daily doses of morphine were discontinued.

Tissues were obtained and fixed from those rats under acute morphinism, at intervals ranging from 1 to 24 hours after injection. In the addicts material was taken at various periods (as indicated in the text), both before and after the daily injections of the drug were discontinued.

The lecithin used was prepared by Merck from eggs; 1 gm. was mixed with 40 gm. of ordinary rat diet of vegetables and bran. A second group of rodents, of precisely the same weight and age as the first, was isolated and fed for a period of 7 months on $4\frac{1}{2}$ gm. of lecithin mixed with 20 gm. of regular food, and given to the animals in the ordinary way.

Glandular Tissues: The glandular tissues studied in this investigation consisted of liver, pancreas, thyroid, submaxillary gland, kidney, stomach and intestine.

Nervous Tissues: The nervous tissues observed were spinal ganglia, Purkinje cells of the cerebellum, motor cells of the spinal cord, as well as the large pyramidal neurones of the cerebral cortex.

* I wish to take this opportunity of thanking Dr. E. V. Cowdry, not only for his kind hospitality, but also for his encouraging interest in this investigation.

Muscle Tissue: The smooth muscular coats of the intestine, skeletal and cardiac muscles were employed.

General Cytological Preparations: For general cytological preparations the tissues were cut into very small pieces in order to ensure rapid penetration of the reagents. Overnight fixation in ice cold Flemming's solution was used, as recommended by Ludford,[2] omitting the 0.5 per cent urea. Sections were stained in iron alum hematoxylin following bleaching with hydrogen peroxide.

Mitochondria: Tissues were treated by Regaud's fixative, in which the sodium sulphate was omitted, and then stained with acid fuchsin and methyl green. This method gave excellent results for all tissues other than brain and spinal cord, for which the Champy-Kull anilin fuchsin technique is recommended. Flemming's solution without acetic acid, followed by staining with Heidenhain's hematoxylin, together with the modifications mentioned by Ma, Lim and Liu[3] was also employed.

Golgi Apparatus: The most successful results were obtained by Ludford's variation of Nassonov's Golgi technique. Instead of staining according to the Volkonsky method, orange G in 95 per cent alcohol was found to give preferable results. In some cases the Nassonov preparations were stained by the Kull method, using toluidin blue and anilin fuchsin. Dehydrating and embedding were always completed in one day, and excellent preparations were obtained by using carbon di-sulphide as a clearing agent. Experiments in times of fixation showed that brain, spinal cord, pancreas and liver render better Golgi bodies after a prolonged fixation of 36 hours instead of the prescribed 24, followed by osmication up to 5½ days at 35° C. Hirschler's modification (1918) was also employed, but the results obtained were more inconsistent than those of the above method. Da Fano's cobalt nitrate (formalin 15 per cent) and Cajal's uranium nitrate techniques were occasionally used. When preparing the reducing fluids for these silver techniques the sodium sulphite was excluded, and the times of fixation for gland tissues were reduced to 3 to 4 hours. Brain and spinal cord, on the other hand, were fixed for 8 to 10 hours.

For studying the mineral organization of the normal and experimental tissues the microincineration procedure, by which the protein compounds of the cells are burned out leaving behind only the mineral residue, was used. The method is similar to that recently described by Policard.[4] Small pieces of tissue were fixed in a solution of 9 volumes of absolute alcohol to 1 volume of neutral formalin. After fixation for 24 hours the material was passed through several stages of absolute alcohol to ensure a complete dehydration and also to remove the formalin. The material was embedded in the usual manner and sectioned at 5 microns. The preparations were then placed on a small quartz slab and transferred to a special electric quartz oven and incinerated at temperatures varying from 625° to 650° C, for periods of 25 to 45 minutes.

The arrangement and deposition of the mineral constituents in the incinerated sections were studied in dark-field illumination obtained by using a Zeiss cardioid condenser. A large sized Spencer Mazda microscope lamp, catalogued as number 394 and equipped with a 500 watt Mazda gas-filled concentrated filament bulb, was used. Directly in front of the bulb was a pair of 4½ inch condensing lenses, which threw a converging beam of light through the cone, illuminating an area about 2 inches in diameter. A pale blue frosted glass, inserted in front of the cone and placed 4½ inches from the substage mirror of the microscope, gave the best illumination for observing the mineral distribution in the incinerated tissues.

The Altmann technique for fixation by drying while freezing, as recently elaborated by Gersh,[5] was also employed for studying the pancreas and liver.* As time would not permit, only normal material and tissue from a rat that had previously received the prescribed single large dose of morphine † were examined by this method 5½ hours after administration of the drug. This procedure consists mainly in freezing the tissue quickly by liquid air prior to dehydration *in vacuo* at −20° C. After embedding in paraffin the material was mounted dry, sectioned at 8 microns and stained by Heidenhain's iron hematoxylin and counterstained in eosin. Tissues thus treated stain extremely rapidly, it being necessary to leave them in the hematoxylin only for 30 to 60 seconds. The advantages of this technique, apart from the rapidity of fixation, are that it should eliminate the possibility of shrinkage, for Gersh contends that when a portion of tissue is maintained at a temperature of −20° C, and dehydrated by means of the Altmann apparatus, the volume of the block of tissue remains unchanged after the process.

III. On the Functional Significance of the Cell Organs and their Behavior During Cell Injury and Disease

Before the effects of morphine poisoning upon the general cytology of the cell can be interpreted it will be well to discuss briefly the possible functional significance of the several cell structures under normal and abnormal conditions.

A review of the literature dealing with the physicochemical nature of mitochondria shows that an enzymatic conception of mitochondrial activity is gradually being realized.[6,7,8] This outlook, from a chemical point of view, is established largely on the investigations of Marston[9] and his co-workers, who conclude, on the basis of their reaction to azine dyestuffs, that mitochondria contain proteolytic enzymes. In addition to this, Robertson,[10] who has carried out an independent study on the function of the lipoid in the mitochondria, has shown that at the phase boundary of the mitochondria and protoplasm synthesis by enzymes may occur. These conclusions derived from the chemical study of the problem receive support when correlated with certain cytological evidence. The assertion of Cowdry,[11] wherein the phase boundary and the surrounding protoplasm of mitochondria are regarded as the seat of processes of

* I wish to express my gratitude to Prof. R. R. Bensley for his hospitality during my visit to his laboratory, and also to Dr. Gersh for kindly fixing the above-mentioned tissues.

† As morphine hydrochloride was not available for this experiment, morphine sulphate was substituted. As far as is known there is no difference in chemical action.

elaboration, commencing with the absorption of molecules of certain solutes, and ending in a series of physical and chemical interactions beween the mitochondria and the incoming substances, is of interest, as this would naturally lead to the production of new compounds of widely different natures. These theories explain, to a large extent, the behavior of mitochondria within the animal and plant cells, where they appear to be closely associated with the production of cellular materials.[12-15]

Another school of thought, however, brings forward evidence to show that the Golgi apparatus alone is associated with secretory processes, and that during this phenomenon the mitochondria play no active rôle. There seems little doubt that the process of fat absorption in the mammalian intestine is correlated directly with the Golgi apparatus.[16] During secretion in the thyroid gland it has been conclusively shown that both the Golgi bodies and the mitochondria are engaged in the process of secretion.[17]

Ma, Lim and Liu,[18] on the other hand, have taken an independent course and conclude that "the Golgi material is the lipoid or fatty component of the mitochondria, which becomes demonstrable on the dissociation of the mitochondrial substance."

Ludford,[19] assuming a broader view of the problem, has taken into consideration the function of the Golgi bodies and correlated it with an enzymatic conception of mitochondrial activity and concludes that synthesis by enzymes may occur at the protoplasmic interface. The resulting products continually diffuse into the cytoplasm, thereby preventing an accumulation at the surface of the mitochondrium, which would inhibit further synthesis. He suggests that these elaborated cell products are concentrated into droplets at the surface of the Golgi apparatus prior to their elimination.

Ludford's theory of a functional interrelation existing between the Golgi apparatus and the mitochondria furnishes an explanation for the behavior of these structures during cellular metabolism, and deserves serious consideration.

Now that the function of these bodies has been considered during normal metabolism it will be necessary to review briefly their response to abnormal conditions and disease. The interesting question arises, and incidentally is still a matter of debate, as to whether or not the reaction of cell inclusions to pathological conditions can be accepted as a diagnostic criterion of disease.

Earlier authors, among whom were Tello,[20] Fananás[21] and Legendre,[22] first reported a definite alteration and behavior of the Golgi apparatus during malignancy, while several others have arrived at contrary conclusions. The general literature in this field is far too conflicting to warrant any detailed discussion, but nevertheless some of the more recent work in relation to the response of these cell organs, following cellular injury and to the administration of chemicals, is of extreme importance when considering the cytopathological action of morphine. In this field experiments of Policard, Garnier and Scott are of interest. The former authors reported that the administration of phlorizin causes a notable decrease in the amount of mitochondrial substance within the tubules of the kidney.[23] Scott,[24] when observing the effect of phosphorous poisoning upon the mitochondria in the acinar cells of the guinea pig, found that these inclusions respond by eventually breaking down into fat granules.

The association of clinical symptoms with functional behavior of the cell organs in severe pathological conditions has been reported. Goetsch[25] has observed a marked increase in mitochondria in many cases of hyperthyroidism, and Homans[26] has correlated mitochondrial changes in the β cells of the islets of Langerhans with diabetic lesions.

The recent work of Ludford[27] on the biological action of X-rays on malignant growths also shows that cytological changes are induced by irradiation. In certain types of transplantable tumors he found that irradiation results in an immediate action on the mitochondria (which become vesicular within 40 minutes), while the Golgi apparatus responds by hypertrophy and fragmentation. Moreover, it is reported that after irradiation enlargement of the cells is accompanied by a definite increase in the mitochondria and an enlargement of the Golgi material.

The physiological investigations of Hall and MacKay,[7] on the effect of administration of glucose on liver cells, show that glucose causes hypertrophy and enspherulation of mitochondria. By these studies they have established a relation between the mitochondria of the hepatic cells and the glucose-glycogen equilibrium. Further evidence is afforded by Weatherford,[28] who, while observing mitochondrial changes in the initial stages of acute inflammation of connective tissue cells, has shown that they give a definite morphological re-

action to this condition and form chainlets, which finally segment into spherules, thus indicating their sensitivity to cellular injury. Nahm [29] has likewise lately shown that the Golgi elements are more readily demonstrated in certain tissues after short autolysis than in the normal.

Although the general literature concerning the changes of these cytological constituents in cell injury and pathological conditions is somewhat conflicting, the evidence as a whole indicates that they are responsive, but at the same time inconsistent in their morphological reactions to such changes. Assuming these cell organs to be the seat of enzymatic action within the living cell, the differences of opinion concerning their behavior under varying conditions might possibly be due to the fact that observations were made during different phases of cellular functioning when their morphological and staining reactions would alter accordingly.

Various authors, when writing of morphine tolerance, point out that there is no substance in the body that will neutralize the poison. Consequently it is natural that one should look for some direct change in the metabolism of the cells, which is quite likely to be morphologically expressed by quantitative and qualitative changes in their cytological components.

IV. General Observations

1. *Acute Morphinism*

Ma,[1] in his dissertation on morphinism, divides the acute reaction to the poison into the stimulated and depressed stages. The former is 1 to 5 hours and the latter 6 to 11 hours after the rodent has been given a single injection. He is able to associate these two periods of the acute condition with accompanying constant cytological phenomena, which are characteristic for each phase. During the first period he reports an increase in the Golgi material, while the mitochondria remain unaltered. In the second, the reverse condition occurs, the Golgi material becoming decreased while the mitochondria respond by a slight increase in numbers.

Detailed examinations of the tissues under acute morphinism, in the present investigation, reveal that, although the cell inclusions in most cases respond morphologically to an acute injection, the results are so variable and inconstant, even in all good cytological

preparations, that it is impossible to associate any alterations with any special phase of the acute condition. In some tissues the cytological demarcation is so slight that it is almost impossible to distinguish between normal and morphinized material. The response, in terms of morphological variations in the cell components during acute morphinism, will now be reviewed.

Liver: An examination of Figures 1–4 demonstrates the general marked differences between the tissue fixed by drying while freezing with the new Altmann technique (Figs. 1 and 2) and the portion of the same liver treated by the ordinary histological procedure (Figs. 3 and 4). The dehydrated tissue shows less affinity for stains and, viewed through the higher powers of the microscope, resembles frozen sections of living tissue. The individual hepatic cells exhibit less shrinkage, and their nuclei, showing less selection for the stain, are larger in proportion to the size of the cell than those seen after histological treatment. The protoplasm presents a granular appearance and the mitochondria, which are preserved, can be faintly distinguished. The endothelium lining the sinusoids of blood vessels is also clearly depicted; the blood cells that stain faintly resemble those observed in the living state, the erythrocytes showing little or no shrinkage.

No gross histological differences could be observed between the acute phase (Fig. 2), 5 hours after injection, and the controls (Fig. 1), beyond a slight hypertrophy of the hepatic cells. The mitochondria and other intracellular components revealed by this technique are too poorly differentiated to observe quantitative or qualitative differences.

Figure 3 depicts a section of liver fixed in Flemming's solution and stained with iron hematoxylin 5 hours after the administration of an acute dose of morphine. A general hypertrophy is likewise observed and is apparent when compared with the normal control (Fig. 4). The hepatic cells following an acute injection invariably show an abnormal number of fat globules (Fig. 3).

Liver fixed from 2 to 10 hours after the last injection, and treated by the general cytological methods, shows a variation from the normal in the number and structure of the mitochondria in the hepatic cells. These changes accompanying the acute condition are found, however, to vary considerably, even in good cytological preparations made from different rats killed at the same time fol-

lowing morphine administration. In certain animals the response to the drug, which involves granulation and hypertrophy of the mitochondria, was so slight that it was difficult to associate any specific alteration with the acute phase. This condition was not observed in all control material, but it is assumed to be a morphological reaction expressing the sensitivity of mitochondria to the poison (Fig. 5).

The Golgi bodies, even in preparations of control liver, exhibit a wide range of structural variation. As the metabolism of this organ is complex, normal and experimental material was obtained at regular intervals after feeding. Even with these precautions it is difficult to establish a definite normal structure in osmic and silver preparations. No fragmentation of the Golgi apparatus is encountered in the morphinized tissue, but a slight hypertrophy, which is inconstant and definitely not specific for any time period following the dose, is occasionally observed.

Examination of nuclear phenomena in controls and acutely affected tissue reveals no deviation from the normal type.

Pancreas: Pancreatic tissue, when treated by the new Altmann freezing method, responds well to this technique. Far less shrinkage is observed, as compared with the ordinary histological controls. The filamentous mitochondria and zymogen granules are detected but are not clearly differentiated from the cytoplasm, thus making quantitative or qualitative estimations impossible. The islets of Langerhans display less affinity for the stain than the acinar tissue. No gross histological differences can be ascertained between normal material and that under acute morphinism.

Control cytological tissue, after fixation in Regaud's fluid and staining with acid fuchsin and methyl green, is represented in Figure 7, where the deeply staining zymogen granules are seen aggregated within the nuclear zone, while the filamentous mitochondria are restricted to the basal regions of the acinar cells. Figure 6 depicts the experimental material, $5\frac{1}{2}$ hours after injection, treated by the above method, and shows clearly an extreme variation in the action of the drug. In most areas the filamentous mitochondria have rounded up into granules which occasionally fuse, forming larger globules. These globules stain poorly and resemble similar structures obtained in the phosphorous poisoning experiments of Scott [24] from the mitochondria of the acinar cells of the guinea pig.

This phenomenon is more marked in some preparations than in others. Examination of a large number of sections demonstrates enormous variations, from a slight abnormality to extreme globule formation. The mitochondria in the islet cells appear to show more resistance to changes. Although the mitochondria in the pancreas of different rats under acute morphinism do not give a constant cytological picture, their general reaction appears to be more specific than in liver cells under similar conditions.

The Golgi apparatus does not show very significant changes when compared with the control material. Slight fragmentation is occasionally met with in the experimental tissue, but no marked increase in the amount of Golgi material, described by Ma during the "stimulated phase," is encountered. Similar variations are observed in tissues 2 hours after an acute administration, at which time the reaction is similar to that produced 5½ hours after the dose. The gross cytological metamorphosis pictured by Ma between these two time periods is not confirmed.

Nervous Tissues: The resistance of mitochondria to pathological conditions in nerve cells, which has been previously commented upon, has been confirmed in this investigation. The cells of the spinal ganglia, anterior horn cells of the spinal cord and the large pyramidal neurones in the cerebral cortex, together with the Purkinje cells of the cerebellum, were examined at various intervals following an acute dose. The mitochondria of these cells of the nervous system display no structural or topographical deviation from the normal. Even in material selected from a rodent that had received a second injection, 3 hours after the first failed, there are no significant alterations. Under these conditions no changes in either the arrangement or staining reactions of the Nissl substance were recorded.

The Golgi apparatus, in contrast with the mitochondria, reveals a variable response to the action of the morphine in the form of a slight hypertrophy and occasional fragmentation. However, the apparatus shows great morphological variation in most nerve cells, even under normal conditions, and varies from rod-shaped bodies to a network formation. This makes it difficult to distinguish any reaction of the neurone to changes in environment of the Golgi apparatus in terms of structural alterations. The hypertrophy and fragmentation is at times most marked (see Figs. 19, 20 and 21) when compared with control material (see Figs. 16, 17 and 18).

These results partly confirm those of Ma, who detected a similar increase in amount during the "stimulated phase" of acute morphinism, but they are contrary to the recent findings of MacEwen and Buchanan,[30] who failed to correlate any deviation from normal in nerve cells under similar conditions. Figures 16 to 18 depict the structure of the Golgi bodies in normal healthy neurones which, in all well controlled preparations, present a broken network of dense anastomosing threads. The apparatus in all larger cells displays a tendency for the network to become dispersed throughout the cytoplasm (see Figs. 17 and 18), while in the smaller cells it appears to be more closely drawn together in a compact mass around the nucleus (Fig. 16). Spinal ganglia, fixed 1 to 5 hours after morphine injection, show that acute poisoning is associated with a slight increase or hypertrophy of the Golgi material. This phenomenon, which is sometimes accompanied by fragmentation of the Golgi bodies, is depicted in Figures 19, 20 and 21. Further observations upon the spinal ganglia during Ma's "depressed period" (10 hours after injection) fail to confirm the contentions of this author. No decrease in the Golgi material is detected in tissues fixed before or after the "depressed period."

Careful studies of a great number of preparations reveal conclusively that acute poisoning is associated with a slight hypertrophy and occasional fragmentation of the Golgi apparatus, the physiological interpretation of which will be a subject of discussion in a later portion of this communication.

Intestine and Stomach: The mitochondria, under normal conditions and during acute morphine poisoning, were extensively studied in the absorbing columnar epithelium of the villi in the small intestine, which lends itself beautifully to cytological investigation. These inclusions within the normal epithelial cells are arranged longitudinally to the axis of the cell, presenting a wavy filamentous appearance (Fig. 8). In the basal or subnuclear region of the cell the mitochondria tend to aggregate and form a capsule-like structure around the nucleus, which is oval in shape and situated in the middle portion of the cell. The accumulation within the subnuclear zone, which consists of granular mitochondria, is apparently a surface tension effect due to their phosphatidal nature.[31] Material fixed after an acute injection reveals small changes in shape and size of the mitochondria. These alterations vary greatly from rod-

shaped structures to globules in preparations taken from different rats at the same time following an acute dose. The most constant feature, in the acute condition, appears to be hypertrophy of the mitochondria. An extreme breaking down and rounding off of these structures is shown in Figures 10 and 11, and contrasts with the normal condition seen in Figure 8. Extensive studies have demonstrated that acute poisoning cannot be definitely associated with any special changes in the form of the mitochondria beyond a tendency for the filaments to break down into polymorphic bodies. It must be pointed out that the mitochondrial structures vary according to the normal functional activities of the cells, and great care must be exercised not to confuse this issue with alterations in shape and size incurred as the result of the drug. It has been shown, for instance, that mitochondria in the epithelial cells of the villi in man alter from the filamentous condition into rod-shaped bodies during fasting. Therefore, it is essential to regulate the diet of the animal as well as the time of collecting the material, before ascertaining any reaction to induced pathological conditions.

The response of the Golgi apparatus to the drug can be observed best in the gastric glands of the stomach, as well as in the glands of Brunner in the submucosa of the small intestine, and can be studied to advantage when sections are cut with a slight obliquity to the long axis of the gland. Under normal conditions the individual cells forming these glands are large, their nuclei being situated in the basal region, while the Golgi apparatus is always seen in the clear area of cytoplasm between the nucleus and the apical region. The mitochondria, when in the filamentous condition, take up a position at the opposite pole of the cell and invariably form a dense mass around the distal region of the nucleus. At the onset of secretory activity the Golgi bodies, which are present in the form of individual polymorphic structures, become reduced in amount and the mitochondrial filaments give rise to granules which migrate with the secretion droplets toward the apical regions of the cell (Fig. 14). This phenomenon has led many workers to believe that the Golgi and mitochondrial substances, by means of chemical interaction, play a dominant rôle in secretory activities, and also stresses the care that should be taken to determine their normal functional variations, before relying on morphological changes as indicators of physiological depression.

Detailed examinations of the cardiac glands of the stomach, pylorus and those of Brunner in the submucosa of the duodenum, obtained at the same time after feeding and at various intervals following an acute injection, reveal that the action of the drug incurs a slight hypertrophy of the Golgi bodies (Fig. 12). This phenomenon is extensively variable and is more marked in some areas of the same preparation than in others, but is not due to imperfect impregnation. In several instances this process is followed by fragmentation of the Golgi material, as illustrated in Figure 12. Extensive studies of the Golgi apparatus under acute conditions of morphinism demonstrate its inconsistent morphological reaction to the poison.

Muscle Tissue: Preparations of skeletal, cardiac and the involuntary muscle in the walls of the intestine show no cytological deviation from the normal type. The apparent fluctuations in the amounts of Golgi and mitochondrial substance, described by Ma, are not encountered.

Thyroid: The cell components of this gland display no cytological response to acute poisoning, beyond an increase in the amount of fat in the vesicular epithelium following a second injection 8 hours after the first. The secretory polarity of the Golgi apparatus, under these conditions, is maintained.

Kidney: A tendency for the short mitochondrial filaments in the epithelium of the convoluted tubules to break down into globules is a phenomenon generally associated with the acute condition. This reaction is more variable even in tissues obtained from the same rodent, and on the whole is most marked 8 hours after injection. The larger globules stain a bright pink with the acid fuchsin-methyl green technique, and various intermediate colors between the red-staining mitochondria and these structures can be seen. Similar staining gradations are detected with the other methods. The Golgi bodies, as far as could be ascertained, are unaltered. But as structural variations under normal functional conditions cover a wide range, the difficulty in associating specific alterations in the form of the apparatus in this organ with induced poisoning is apparent.

The cytological changes produced by the action of morphine, described above, were not found to be similar in different tissues, as Ma contends, nor could any abnormal nuclear phenomenon be attributed to the morphine.

The behavior of the rats to acute doses of morphine hydrochloride appears to be a controversial point. Ma holds that they show symptoms of slight irritability, while MacEwen and Buchanan [30] report that their rodents, after a similar dose, pass into a deep narcosis for several hours, after 3 to 5 minutes following the injection. My observations, however, entirely confirm those of Ma.

2. *Chronic Morphinism. Cytopathology of Chronically Morphinized Tissues Examined at Intervals Before Discontinuation of the Drug*

When discussing the pathology of morphine tolerance Ma contends that, provided the daily dose is administered regularly, no cytological deviation from the normal occurs, although he describes certain fluctuations in the form of specific rhythmic changes in both amount and form of the cell components during the interval between injections. During what Ma terms the "middle of the craving period satisfied," which is 12 hours after the last injection, a general dispersal and increase in the Golgi and mitochondrial material is reported. It is further contended that microscopic preparations obtained from rats at the end of 24 hours, before they receive their next regular dose of morphine, contrast cytologically with those of 12 hours earlier. In the interim a decrease in the amount of Golgi material occurs and the inclusions gradually return to their normal morphological state.

The evidence derived from this investigation does not confirm that of Ma. Certain slight changes in the morphology of the cell organs, which were by no means specific to all tissues, nor to any period either before or after the regular injection, were observed.

Examination of tissues taken from rats that were under chronic morphinism for 6½ to 7 months revealed the following cytopathological changes.

Stomach and Duodenum: Figure 13 represents a transverse section through a gland of Brunner in the submucosa of the duodenum. In the vast amount of material studied the Golgi bodies in these gland cells possess a depleted appearance and contrast with similar tissues under normal and acute conditions (compare Fig. 13 with 12). This picture reveals an extreme variation of the apparatus in the chronic state, and also shows the loss of power for selective staining

as well as morphological change. Both in the normal and acute phases it tends to exist as a compact polymorphic body situated within the apical zone of the cell. In the chronically morphinized tissue, however, the depleted Golgi substance varies from small irregular bodies to anastomosing threads, which do not confine themselves entirely to the apical regions, as in the control preparations, for they are frequently detected lying around the basal aspect of the nucleus (Fig. 13).

The reaction of the mitochondria to chronic poisoning is demonstrated clearly in the absorbing columnar epithelium of the villi. These structures are usually filamentous and orientated longitudinally to the axis of the cell in normal material (Fig. 8). In chronically morphinized tissues (see Fig. 9) they are extremely variable, tending to become fragmented, forming irregularly shaped bodies, somewhat depleted in appearance and finally losing their polarity in the cell. It appears likely that these phenomena, especially the loss of polarity, are due to chemical alterations in the structure of the mitochondria.

Cowdry[32] has admirably summarized the evidence for concluding that the cytoplasmic organs are sensitive to pathological conditions, and the cytological deviations from normal occurring in these gland cells are further confirmation of this sensitivity. Slight departures from the normal up to the extreme condition pictured in Figure 9 are common in this chronic material, taken at any period between the regular injections. This again demonstrates forcibly the enormous limits of structural variation which make it impossible to link any specific change with any special degree of chronic poisoning.

Nervous Tissues: Owing to the complex and diverse morphology of the Golgi apparatus, even under normal conditions, it is only with difficulty and caution that any cytological changes can be associated with any degree of morphine tolerance in nerve cells. Beyond an aptness for the apparatus, in well controlled preparations, to display less affinity for the several stains, no abnormality was met with.

The curious resistance of mitochondria to pathological conditions in neurones is again confirmed by a study of their behavior in the chronically morphinized material.

Pancreas: In many preparations of this tissue small departures from the normal in the cell components, which were confined to the

experimental material, are encountered. The mitochondria retain their normal filamentous appearance. This lack of response is not found in the case of acute poisoning (Fig. 6). At the same time they show less absorption for the stains, a phenomenon that appears to be characteristic of the chronic state. Slight fragmentation of the apparatus is occasionally met with, but the marked increase and dispersal of this substance within the apical portion of the acinar cells, in the form of small granules, reported by Ma, 12 hours following the last morphine administration, is not apparent.

Thyroid: Certain interesting pathological changes in this gland appear to be associated with the chronic poisoning. The vesicles, in the majority of cases, contained visibly smaller amounts of colloid, a phenomenon that becomes significant when compared with glands from control rodents. The cytological constituents retain their normal morphology and staining reactions. The accumulation of fat characteristic of the acute condition is not detected, and the lipoid droplets generally seen in the epithelium of the healthy thyroid are entirely absent. The interstitial connective tissue shows no significant variations.

Kidney: The mitochondria present in the renal epithelium of the convoluted tubules are well developed and resemble those seen in the normal organ, except that in certain areas of most preparations the same inability to absorb the stains is met with. Globules, similar to those detected under acute morphinism, are present in a modified degree. The Golgi bodies display an irregular behavior by fragmenting in certain localized areas in the epithelial cells, and come to resemble the structures pictured by Ma during the middle of the "craving period satisfied" of chronic morphinism.

Liver: Preparations of the liver show the mitochondria present in a depleted form, otherwise their morphology and distribution within the hepatic cells appear normal. Variable decreases in the glycogen content of these cells is a conspicuous feature in many regions of chronically morphinized liver.

In view of these results it is of interest to review the observations of several authors in this field. Arnold [33] was one of the earlier workers who found an association of glycogen formation with mitochondrial activity. This work is not in accord with the later contentions of Bang and Sjövall,[34] who found that the distribution of glycogen within the cell protoplasm does not conform to the

distribution of the mitochondria. The previous observations of these authors were later confirmed by Mann [35] in 1928, who likewise discovered no evidence of a morphological relation between the mitochondria and the glycogen of liver cells. Kater [36] in a recent paper has come to the conclusion that there is no evidence for associating the deposition of glycogen in the liver cell with mitochondrial activity. In view of this conflicting evidence it is impossible to determine whether the depletion of the mitochondria, characteristic of liver under chronic morphinism, can be correlated with the decreases in glycogen content. The Golgi material failed to show any reaction to chronic poisoning.

Muscle Tissues: Skeletal and cardiac muscles reveal no significant cytopathological changes that can be attributed to the effect of continuous morphine administration.

3. *Behavior of the Golgi and Mitochondrial Material in Addicts, After Abrupt Withdrawal of the Drug*

The "craving period" of chronic poisoning after withdrawal of the drug is divided by Ma into two phases, the first 1 to 4 days and the second 4 to 6 days after the final injection, and he finds that each period is definitely indicated by some cytological change. The first phase of the "craving period" is marked by such a decrease in the amount of Golgi substance that, in some cells, he reports it as almost absent. The second period is marked by a reappearance of the Golgi material and an increase in the mitochondria, which are enlarged and display better affinity for the stains. At the end of 10 to 12 days these inclusions return to their normal state.

Detailed microscopic examination of the form and fluctuation of the cytological constituents in the tissues of the addicts during these phases reveals that the morphological variations encountered are not necessarily typical of any special state. They are found to exist in different areas of preparations from the same piece of chronically morphinized tissue, and therefore come within the limits of possible variations of the Golgi apparatus and mitochondria in this material. Similar limits of variation may be found occasionally in the cell components in any tissue, even in the normal state.

Observations were made upon the tissues of addicted rats at different intervals from 24 hours until 3 weeks after sudden discon-

tinuation of morphine. Although it is known that abrupt withdrawal leads to severe pathological conditions such as edema, in many tissues, this investigation has shown, contrary to Ma, that it is impossible to correlate any specific cytological state with these phenomena, and no marked cytological demarcation in the form of a sudden decrease of the Golgi lipoid (as Ma terms it), even in preparations 48 hours following discontinuation, was detected. Edema incurred by morphine withdrawal, according to Barbour, Hunter and Richey,[37] is accompanied by rapid loss of lipoid in most tissues. This should correlate theoretically with Ma's findings, when taking into consideration his chemical interpretation of the lipoidal structure of the Golgi apparatus.

Even in tissues such as the gastric glands of the rat of 6½ to 7 months addiction, in which the cell components appear to react to the drug in terms of a variable depletion of the Golgi material, no conspicuous cytological differences can be detected at any interval immediately following withdrawal. The same is found to be the case in tissues that give no significant response to continuous administration of the poison.

Extensive studies have shown that the limits of morphological variation of the cell inclusions seen in the same piece of chronically morphinized tissue are almost identical with those Ma obtained in any particular phase, either during or after the period when the regular morphine injections were withheld.

4. *Microincineration of Normal and Experimental Tissues*

The location of the mineral constituents in normal, acute, and chronically morphinized tissues both before and after withdrawal, as revealed by the method of microincineration of Policard,[38] was extensively investigated. After burning away the protein compounds the distribution and orientation of the inorganic salts were studied in dark-field illumination.

Although thorough examination of the incinerated material shows a specific distribution of the inorganic residues, which are visibly constant in cells forming similar tissues, no marked departures from the normal are observed between the experimental and control material.

Certain abnormal ash distributions are, however, occasionally met with, but repeated experiments demonstrate that this phenome-

non is due, in all probability, to an artificial clumping of the mineral constituents brought about during the process of burning — a factor that appears to be largely dependent upon the temperature and time of incineration.

Nevertheless, definite changes in the amounts and localization of mineral salts in various pathological states have been reported.[39, 40, 41] The most striking results, in view of the negative ones obtained in this investigation, are those of Policard, Noël and Pillet.[42] These authors comment upon the effect of different diets on the mineral distribution in incinerated liver sections from white mice. Variations from the normal were found after feeding the rodents on strict sugar, protein and fat diets, and also in the rate at which these tissues incinerated.

This significant mineral variation, resulting from the changes in diet, observed by these authors, is interesting as no inorganic disturbance, following a lecithin diet of rodents for long periods, was detected by incineration. In the case of lecithin feeding this might be expected, for Glikin,[43] Burow,[44] Altschul,[45] and Stern and Thierfelder [46] concur that iron and calcium are present in lecithin in appreciable amounts.

The administration of lecithin is followed by an increase in the lecithin content of the liver,[47] but incinerated sections of this organ from rats fed for over 4 months on a lecithin diet fail to reveal any differences in mineral localization when compared with liver obtained from control rodents fed on a regular diet.

Appreciable variations in the amounts of inorganic salts in normal and corresponding pathological tissues estimated by chemical analysis have likewise been described. The most noteworthy researches in this sphere are those of Eaves,[48] who gives interesting data concerning the increase in mineral salts during many pathological conditions in the human brain, and that of Adams,[49] who discovered that serum calcium is higher in the blood of rabbits suffering from senile cataract than in normal animals of the same age.

As it has been shown that well advanced morphine addiction not only causes dehydration in certain internal organs, but that sudden withdrawal of the drug results in a remarkable redistribution of water and severe pathological conditions such as edema of the brain, liver, kidney and muscles, as well as a marked increase in blood calcium, one would naturally expect such changes to be indicated by

a disturbance of the mineral organization of the cells. Microincinerated preparations, however, of liver, kidney and brain, which were pathologically affected by sudden morphine withdrawal, show no deviation from the normal controls. Even if the pathological changes incurred by morphine poisoning are inorganically expressed by small specific fluctuations of the mineral constituents, it is unlikely that any such slight variations could be detected by the present microincineration technique, although a recent paper by Scott[50] on quantitative estimations of the ash in incinerated preparations indicates possibilities in this direction.

5. *The Cytological Effect of Lecithin Feeding on Normal Tissues, and on Acute and Chronically Morphinized Tissues, both Before and After Withdrawal of Morphine*

The biological significance of lecithin feeding in normal animals, as well as its effect upon the toxicity of morphine and other alkaloids, has been a subject of much controversy. That lecithin administration results in an increase in the general body weight and acts as an agent stimulating normal growth is a matter of debate.

Ma, when investigating the physiological results of lecithin feeding on the influence of body weight in white male rodents, reported a significant increase. After one month his experimental animals showed in some instances an increase of 9 gm. over the controls. A still greater increase of 27 gm. was found in the lecithin-fed females, as compared with similar females of the same age maintained on the control diet.

Ma attempts to correlate this increase in weight, following yolk-lecithin feeding, with definite cytological phenomena. His preparations showed that the cell components are better developed in tissues from normal lecithin-fed rats, the most marked differences being exhibited by the Golgi material. Ma's physiological interpretation of this phenomenon is based on the supposition that the Golgi substance consists mainly of lipoids, among which lecithin occupies the first place. The fact that lecithin is not absorbed as such will be discussed later.

The growth experiments with lecithin feeding, in this investigation, yielded negative results. Twelve rats (6 males and 6 females) of the same age and weight were segregated and fed for 4 months on

Merck's yolk-lecithin, in proportions as indicated in the text. At the end of this period their weights were compared with corresponding controls fed on the regular diet, and no significant increases in either their growth or weight were recorded. It might be suggested that the vast differences obtained by Ma between his lecithin and control-fed rodents are explicable on the grounds that the control diet was inadequate. Slight variations in body weight, however, were detected between the chronically morphinized rats of 6½ months addiction and those fed upon an ordinary diet. The loss of weight in these addicts was more marked after the first 2 months and on an average never exceeded 4 to 5 gm. Whether this was directly due to the pathological action of the morphine, or secondary effects, associated with a significant loss of appetite, is difficult to ascertain. After 4 months of morphine administration these rodents developed a normal appreciation of food, which was followed by a rapid increase in body weight. It is interesting to record that Barbour, Hunter and Richey,[37] when observing the reaction of dogs to morphine addiction, found that the animals always refused their food during the commencement of a series of injections.

Microscopic examinations of control tissues again demonstrate the enormous limits of cytological variation, particularly expressed by varying amounts of the Golgi substance in normal material. These variations make it exceedingly difficult to determine any relative increase of the cell components in the experimental tissues that might be incurred as the result of lecithin feeding.

Another important factor to be taken into consideration when making quantitative estimation is the metabolic state of the cell at the time of examination. Figure 14 depicts a transverse section through the gastric gland of a lecithin-fed rat killed at the onset of secretory activity, in which an increase in mitochondria and associated granules, together with a decrease in Golgi substance, is involved. Observations on such material during the resting stage of glandular activity and also in other tissues show nothing significantly above normal. The cytological constituents in lecithin-fed tissues are always clearly rendered, and in several instances better developed than in the controls, but as this occasional phenomenon is an irregular one it cannot be attributed solely to the lecithin diet.

In order to restore the Golgi apparatus, which Ma found "almost entirely lacking" during the "craving period," following morphine

withdrawal, he contends that if lecithin is regularly administered together with the usual dose of the drug a few days before discontinuation, the Golgi material is thus prevented from becoming reduced and so retains its normal appearance. He shows cytologically that without lecithin treatment the cell inclusions revert to their normal condition 10 to 12 days after the final injection, but that lecithin feeding, on the other hand, 6 days before and after, prevents this abnormal reduction of the Golgi substance.

As material studied in this investigation reveals that the apparatus, and in some instances the mitochondria, in chronically morphinized tissues undergo a depletion before withdrawal (see Fig. 13), it is difficult to compare the results of subsequent treatment with those obtained by Ma. Experiments, nevertheless, were carried out in this direction, but no significant results were observed. Chronically morphinized tissues from rodents, one-half of which were control-fed and the other lecithin-fed, 8 weeks before morphine discontinuation, were examined 2 days after sudden withdrawal, and no cytological differences beyond the normal morphological variations typical of any healthy cells were observed.

Figure 15 represents the form of the Golgi apparatus, typical of the gastric glands, of a 6½ months addict, lecithin-fed and killed 4 days after withdrawal of morphine. The apparatus has in no way responded to lecithin treatment. It is still reduced in amount, having a slightly depleted appearance, and in no way differs from that of the control-fed material obtained under similar conditions. Extensive examination of various tissues yields no significant results.

The cell inclusions that reacted to the chronic poisoning in the several organs gradually return to their normal state within 2½ weeks after the final injection, which confirms the previous observations of Ma.

The immediate effect of an acute morphine injection upon the Golgi material, of nerve and glandular tissues of a normal lecithin-fed rodent, shows that beyond a slight hypertrophy of the apparatus, frequently observed under such conditions, no cytological deviation from type is detected which would suggest any interaction between the alkaloid and the lecithin.

V. Discussion

A review of the behavior of the several cell organs during acute and chronic poisoning, as well as their reactions to cell injury and disease, shows that they are responsive to gross metabolic and pathological changes, but their use as indicators of fine degrees of physiological depression is not based on sufficiently reliable evidence. When taking into consideration the wide range in the limits of morphological variation of the inclusions, found even in normal healthy cells, it is impossible to correlate any slight structural alteration with any specific degree of morphine tolerance, and on these grounds this investigation fails to concur with the results obtained by Ma. The Golgi elements, in particular, have been shown to be inconstant morphological units, their structure depending largely upon their reaction to the type of technique employed. The apparatus, as seen even in well controlled preparations, in the same piece of tissue treated by either the osmium and silver methods, invariably shows structural diversity.

These experiments also demonstrate that the appearance of the Golgi elements is not only contingent upon the impregnation, but also the period of fixation which had to be regulated according to the type of tissue. Some of the variable factors controlling the impregnation of the Golgi material have recently been discussed in detail by Nahm.[29]

The mitochondria appear to be more consistent in their reactions to different cytological procedures and to induced abnormal conditions. Their extraordinary resistance to acute and chronic poisoning in nerve cells is difficult to interpret, especially when variable morphological changes are associated with this phenomenon in other tissues. These results confirm those of Strongman [51] and McCann,[52] the latter finding that mitochondria in experimental poliomyelitis remained unchanged even after the nerve cells had undergone partial degeneration, while the former demonstrated their immutability in functional exhaustion.

The hypertrophy of the cell organs following administration of an acute dose of morphine, which is very noticeable in the Golgi elements of the nerve cells of the spinal ganglia and in the mitochondria in the acinar cells of the pancreas, is a difficult phenomenon to explain in view of the uncertain chemical composition of these

inclusions. Bancroft and Richter,[53] when inquiring into the physiological action of anesthesia on tissues, suggested that narcotics such as morphine caused an aggregation of the cell colloids. This is worthy of consideration and the possibility that an absorption of the alkaloid might lead to an increase in the surface area of these cell constituents should not be overlooked.

The depletion of the Golgi and mitochondrial material, expressed variably in terms of partial loss of staining reaction combined with a slight decrease in their bulk, typical of most chronically morphinized tissues, is an interesting gross cytological response that might possibly be correlated, in some respects, with the physiological observations of Barbour, Russell, Flowers, Dunham and Hunter.[54] These investigators find that well advanced morphine addiction incurs dehydration of certain of the internal organs, and also that sudden withdrawal of the morphine causes edema, with loss of lipoids, in some parts of the brain, liver and kidney, together with a remarkable redistribution of water.

Assuming that the generally accepted idea of the lipoid structure of the Golgi substance is correct, this might account for the depletion of the apparatus after morphine discontinuation, but as it seems to take place in certain tissues of well advanced addicts, before the drug is withheld, it is difficult to interpret.

Barbour, Hunter and Richey [37] report an interesting observation in this connection. They discovered that when fat-fed dogs were suddenly deprived of morphine a rapid loss of liver fat (in 4 days diminished by 36 per cent in some cases) occurred, while the livers of dogs on a non-fat diet showed an increase. This observation is of interest in view of the recent work of Smith [15] and Kater and Smith,[13] who show that mitochondria in liver cells probably act as catalysts, stimulating a synthesis of fat from the fatty constituents of the cell. It is therefore possible that the reserve fats of adipose tissues in the dogs fed on a non-fat diet are conveyed by the blood stream to the liver cells and here resynthesized by mitochondrial activity.

Overton [55] finds that the power to produce narcosis is entirely dependent upon the power of the substance to dissolve the lipin membrane of the given cell. As it is seen that the cytopathological response to morphine varies according to the tissue acted upon, it might be suggested that this difference in effect is produced by the

fact that the chemical structure of the cell membrane varies with certain types of tissues and gives a different response to morphine in each case.

The results presented in this paper on the negative effect of lecithin administration in normal, and in chronically morphinized rodents, both before and after morphine withdrawal, become more explicable after reviewing the evidence of several workers in this field.

Lecithin, which belongs to the group of phospholipoids, was first isolated by Gabey in 1846, and on hydrolysis yields glycerophosphoric acid, fatty acids and choline.[56] Following its discovery it was used clinically, and later abandoned, as a cure for diseases ranging from beriberi and scurvy [47] to anemia and debility,[57] and its influence as a growth-stimulating factor is still a matter of controversy. Ma,[58] however, reports success in applying this substance to suppress symptoms during treatment of human opium addicts, and also finds that on microscopic examination of their blood the Golgi apparatus in leukocytes, during the "craving period," decreases considerably in amount, and increases provided the patient is treated with lecithin. In a later investigation [59] he finds that the erythrocytes of chronically morphinized rats "received great benefit from the treatment with lecithin after withdrawal."

This last work of Ma is of interest in view of the recent observations of Masasue,[60] and Grönberg and Lundberg.[61] Masasue finds that blood taken from human morphine addicts, when compared with that of normal persons, shows a lower red count. Grönberg and Lundberg have found independently that lecithin administration *in vivo* brings about a rise in the number of red blood cells. From this evidence it might be assumed that morphine has a depressing effect while lecithin stimulates erythropoiesis in bone marrow. It has been shown further that, in the case of advanced human morphine addicts, a single injection of the drug results in a leukocytosis within 30 to 60 minutes. As this change of leukocytosis, however, is only a transient phenomenon (the blood becomes normal in 90 to 120 minutes following the injection), it is difficult to correlate this physiological change with the cytological changes described by Ma,[59] as occurring in the blood of chronically morphinized rats for extended periods after withdrawal.

MacLean,[47] in his monograph, "Lecithin and Allied Substances," admits that the results of feeding experiments with lecithin are in-

conclusive, and that its administration does not appear to be followed by very significant results. Other authors point out that owing to the unsatisfactory state of knowledge concerning the lipins it follows that their exact function in animal economy must necessarily be obscure.

Ever since Danilewsky in 1895 published his startling results in which he found that tadpoles placed in water containing 0.07 per cent lecithin gained 300 per cent more weight than controls kept in ordinary water,[62] many workers have come forward with most conflicting evidence concerning the growth-stimulating powers of this substance. Ma's results entirely confirm those of Hatai,[63] who discovered that white rats, which received lecithin either by injection or mouth, gained in body weight more rapidly than the control-fed animals; the gain in the experimental rodents being on an average 60 per cent. Likewise, Desgrey and Zakey [64] describe an increase in the body weight, especially in the nervous and skeletal systems, following injections of lecithin. It is interesting to note that these conclusions were not entirely confirmed by Hatai, who further found that administration by mouth incurred greater gain in body weight than by inoculations. Then Goldfarb [65] reported that there was no clear evidence of growth stimulation as a result of administration of this substance. He entirely failed to confirm Danilewsky's work on tadpoles, and after extending these experiments to both carnivorous and herbivorous mammals failed to concur with the previous results of Hatai, Desgrey and Zakey.

A more recent publication on this subject by Brachiesi [66] gives an independent view on the nature of lecithin stimulation of growth, which differs from the findings of Ma and other authors. Brachiesi treated guinea pigs with subcutaneous injections of lecithin for 15 successive days. The animals were killed after 18 days. The lecithin-fed animals showed a decided increase in weight over the controls up to the 12th day. Their weight then became constant for the next 6 days. From these experiments this author assumed that lecithin modified the metabolism up to the 12th day and that afterwards an equilibrium was reached. Other groups of animals were similarly treated over longer periods and the same phenomenon was encountered.

There is, in fact, evidence that suggests the nature of the influence of lecithin on growth is rather inhibitory. Robertson [67] found that

large doses of lecithin given to mice produced a slight retardation of growth. Similar retardation in suckling mice, following the administration of 100 mg. of egg-lecithin per day, given by mouth to the mother, was recorded by Robertson and Cutler.[68]

This result is supported by the evidence of Fingerling [69] who contended that the addition of lecithin to the diet is without effect on the secretion of milk.

The specific nature of the fatty acids of lecithin was first doubted by McCollum, Halpin and Drescher,[70] who demonstrated that the degree of unsaturation of the lipin fatty acids of egg yolk is accidental and can be easily influenced by diet. If this is so it might help to explain many of the conflicting results on the growth phenomena in relation to this substance obtained from eggs.

In view of the results of these workers and those obtained during this investigation the following experiments of MacArthur and Luckett [71] are of interest. These investigators succeeded in eliminating not only lecithin but cephalin, cholesterol and fat from the indispensable constituents of foods for mice, and Osborne and Mendel [72] found that the body weights in rats could be maintained on a fat-free diet.

The recent cytological findings of Ikeda [73] on the effect of lecithin on the Golgi and mitochondrial elements in chicken material do not agree with those of Ma, and the present investigation. This author reports that lecithin inhibits the development of the Golgi apparatus, but has the opposite influence on the mitochondria. The results of Okada,[74] on the effects of lecithin upon the mitochondria in the neurones of rabbits, confirm those of Ikeda.

When reviewing the several conflicting observations of these investigators the fact that lecithin is not absorbed as such raises a question which Ma admits is difficult to answer. MacLean [47] showed that it is acted upon by lipase within the intestine and broken down into fatty acids, glyceryl-phosphoric acid and choline. This may also have a bearing in some degree on the negative results obtained by lecithin administration, both as a growth-stimulating substance and a therapeutic agent for the treatment of morphine addiction in rats.

The histological changes in the thyroid gland under acute and chronic morphinism, recorded in this study, do not coincide with those of Scarborough.[75] This worker finds that chronic poisoning

is not associated with any change in the structure of this gland and, moreover, that the combination of chronic morphine poisoning with thyroid feeding inhibits the action of the drug upon the nervous system and checks the edema of the internal organs, generally associated with this condition.

When correlating cytological changes with pathological depression the fact that the cell inclusions are morphologically conditioned according to the chemical state of the cell must first be considered. As has been shown, the reduction of the Golgi material, enspherulation and increase of the mitochondria, are all part of a normal phenomenon associated with the general functional behavior of the gland cells during secretory activity. Even in healthy nonglandular cells, in both the animal and plant kingdoms, fluctuations in the amount of the inclusions, which are probably morphological indications of a synthesis involving condensation of material within them or at their surfaces, are frequently encountered. As physicochemical processes within the cell appear to be largely responsible for the enormous limits of structural variation of cytoplasmic constituents, seen in normal tissues, these experiments have emphasized the danger of relying on these variations for gauging fine degrees of physiological change in cells undergoing diverse chemical influences.

VI. Summary

1. An investigation has been made of the reactions of cytoplasmic structures in different cell types to varying degrees of morphine poisoning, during the periods of regular injections of the drug and at stages following its abrupt withdrawal.

2. Under conditions of acute morphine poisoning the cell inclusiens show variable morphological changes, which are described in detail, but specific changes, indicative of special phases of the acute condition, could not be recognized consistently.

3. More significant, but still inconstant, alterations in the cytoplasm follow addiction for periods of 6 months or more and before the injections are discontinued. In the glands of the stomach and duodenum the Golgi apparatus is depleted and the mitochondria are frequently fragmented and without regular polarity. Minor changes are recorded in the pancreas, thyroid gland and liver.

4. Abrupt withdrawal of morphine, after a prolonged period of addiction, has been shown by other investigators to cause patho-

logical conditions in various tissues. There is no evidence, however, of cytoplasmic alteration which would indicate the effects of withdrawal of the drug.

5. Morphine poisoning does not cause alterations in the mineral constituents of tissues that can be detected by microincineration, although this technique has been successful in the investigation of various common pathological conditions.

6. The growth-stimulating action of lecithin fed to normal rodents is not confirmed, nor does this substance appear consistently to alter the cytoplasmic structures in normal tissues. Lecithin administered therapeutically to acutely and chronically morphinized animals, both before and after withdrawal of the drug, has no significant cytological effect which would suggest that there is any interaction between itself and the morphine.

7. The pathological action of morphine on cells in various tissues appears to occasion changes in the cytoplasm that are so delicate and variable that morphological alterations in cell structures, such as the Golgi apparatus and mitochondria, are not reliable indicators of the acute, chronic and withdrawal phases of morphinism.

8. Morphological changes in the cell organs are met with in special instances following morphine poisoning, but when the limits of variation of the cell organs, which alter even in different functional states of the normal healthy cell, are recognized, these changes are found to range within such limits and therefore lose significance as indicators of the different phases of morphine poisoning.

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DESCRIPTION OF PLATES

PLATE 71

FIG. 1. Control section of liver from a normal healthy rodent after treatment by the Altmann technique for fixation by drying while freezing, stained with Heidenhain's hematoxylin and counterstained in eosin. The hepatic cells show less shrinkage when compared with those of a portion of the same liver, fixed by the ordinary histological procedure, and their nuclei show less affinity for the stain.

FIG. 2. Showing section of liver, fixed by the Altmann method, 5 hours after a single acute injection of morphine sulphate. Observe the slight hypertrophy of the tissue.

FIG. 3. Same material morphinized as in Fig. 2, but fixed in Flemming's solution and stained by Heidenhain's hematoxylin instead of by the Altmann method. Note the accumulation of fat in the hypertrophied hepatic cells, which is a phenomenon associated with the acute condition.

FIG. 4. Showing section of control liver obtained from a normal healthy rodent, after treatment by the ordinary histological procedure. The differences in staining as well as the general condition of the cells are apparent when compared with similar material treated by the Altmann technique, as demonstrated by Fig. 1. Note the absence of cell hypertrophy and fat accumulation typical of acutely morphinized liver.

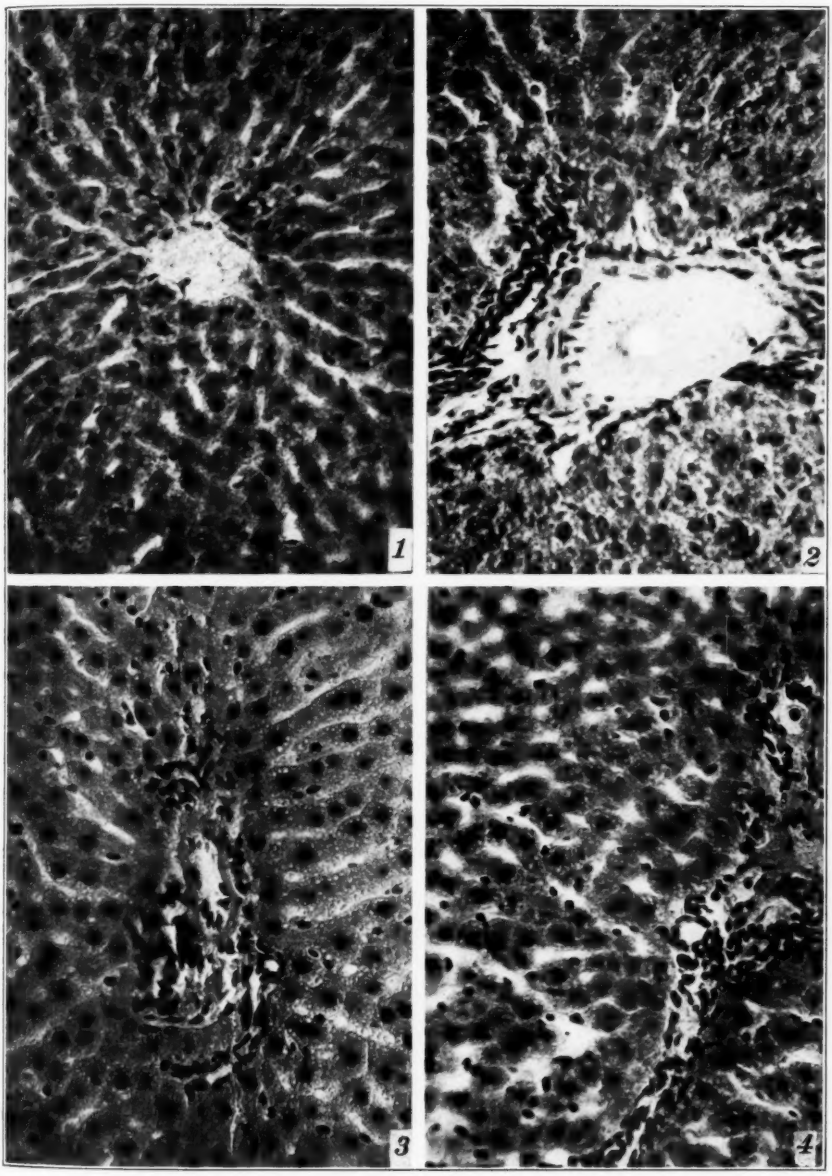
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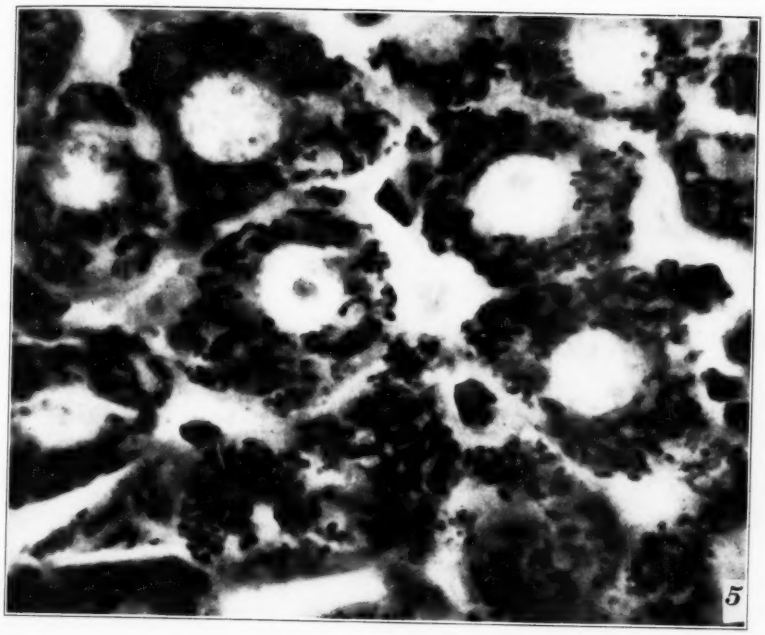
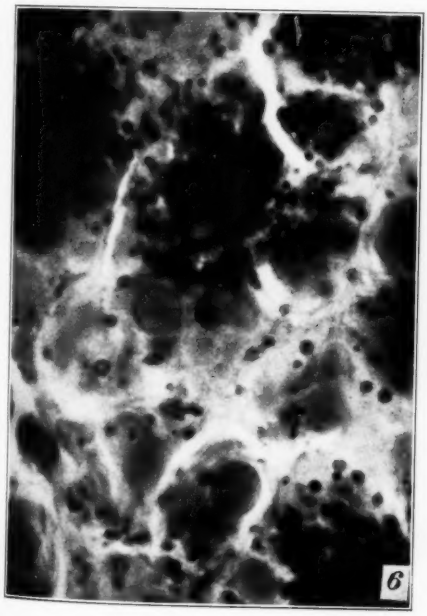
PLATE 72

FIG. 5. Liver fixed 8 hours following an acute injection of morphine hydrochloride. This photomicrograph shows an extreme variation of the reaction of the mitochondria to the poison. The material was fixed in Regaud's fluid prior to staining with acid fuchsin and methyl green.

FIG. 6. Depicting an extreme variation of mitochondrial behavior in the pancreas $5\frac{1}{2}$ hours following an acute injection of the drug. Observe the formation of lightly staining globules and the absence of filamentous mitochondria. Material was fixed and stained as above.

FIG. 7. Control section of pancreas from normal healthy rat fixed and stained as above. Note the mitochondrial filaments are restricted to the basal region of the acinar cells and also the absence of globules.





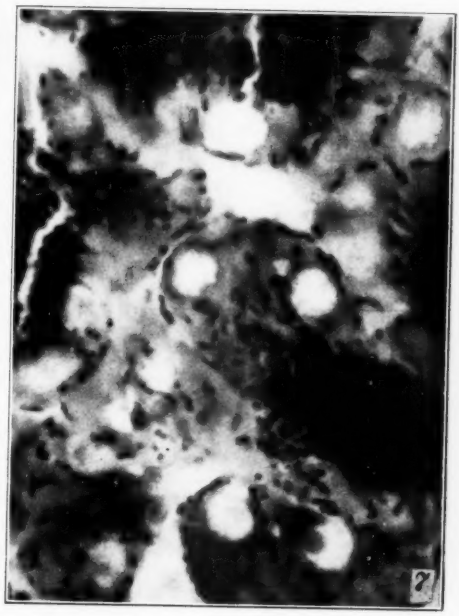


PLATE 73

FIG. 8. Showing filamentous structure of mitochondria as seen in the absorbing columnar epithelium of the intestinal villi in a normal rat after fixation in Regaud's fluid and staining by acid fuchsin and methyl green. Note the longitudinal arrangement of mitochondria to the axis of the cell, together with the basal or subnuclear aggregation of mitochondria forming a capsule around the nucleus.

FIG. 9. Demonstrating the reaction of mitochondria to chronic poisoning in the epithelium of the intestine of a $6\frac{1}{2}$ months morphine addict. The mitochondria have a slightly depleted appearance in contrast with control in Fig. 8. Material fixed and stained by Ludford's variation of the Nassonov technique.

FIGS. 10 and 11. Revealing an extreme reaction of mitochondria to a single massive injection of morphine hydrochloride. In this material the mitochondria were seen to vary from chainlets to isolated spherical bodies within the same preparation. In these figures the mitochondria are seen as rounded hypertrophied structures. Compare with control formation in Fig. 8 and the chronic condition as demonstrated in Fig. 9. Material fixed and stained as in the latter.

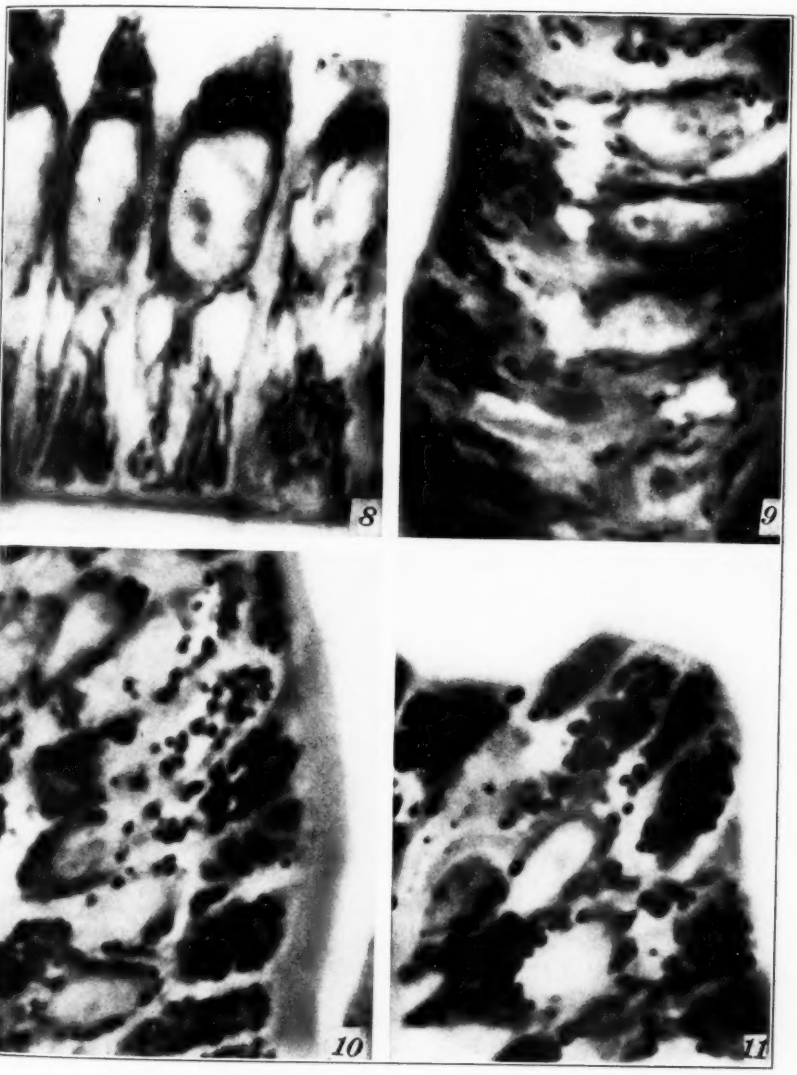

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PLATE 74

FIG. 12. Transverse oblique section through gastric gland of stomach in rat killed 7 hours following a single large dose of morphine. Observe slight hypertrophy and fragmentation of Golgi bodies. Material treated by Hirschler's modification (1918).

FIG. 13. Showing depleted appearance of the Golgi substance in a transverse section of a duodenal gland of Brunner in the submucosa, obtained from a rat of 7 months addiction before withdrawal of the morphine. This photomicrograph shows an extreme variation of this condition and contrasts with the condition of the Golgi apparatus of acutely morphinized material, as seen in Fig. 12. Material fixed by Ludford's variation of the Nassonov technique counterstained by orange G in absolute alcohol.

FIG. 14. Section cut transversely through a gland of Brunner of a normal lecithin-fed rat, killed during secretory activity. The Golgi bodies have become reduced, the mitochondria are granular and have migrated together with secretory droplets toward the apical region of the cell; this phenomenon is not due to lecithin feeding, and demonstrates the cytological state of a gland during normal functional behavior. Tissue fixed by Ludford's variation of Nassonov's technique, counterstained with orange G.

FIG. 15. Showing the cytological appearance of a single gastric gland of a lecithin-fed rat of 6 months addiction, killed 4 days after abrupt withdrawal of morphine. The Golgi material is rendered clearly in the apical region of each cell in the gland. The Golgi substance still retains its depleted appearance peculiar to the chronic condition, and has not responded to lecithin administration. Tissue fixed and stained as in Fig. 14.

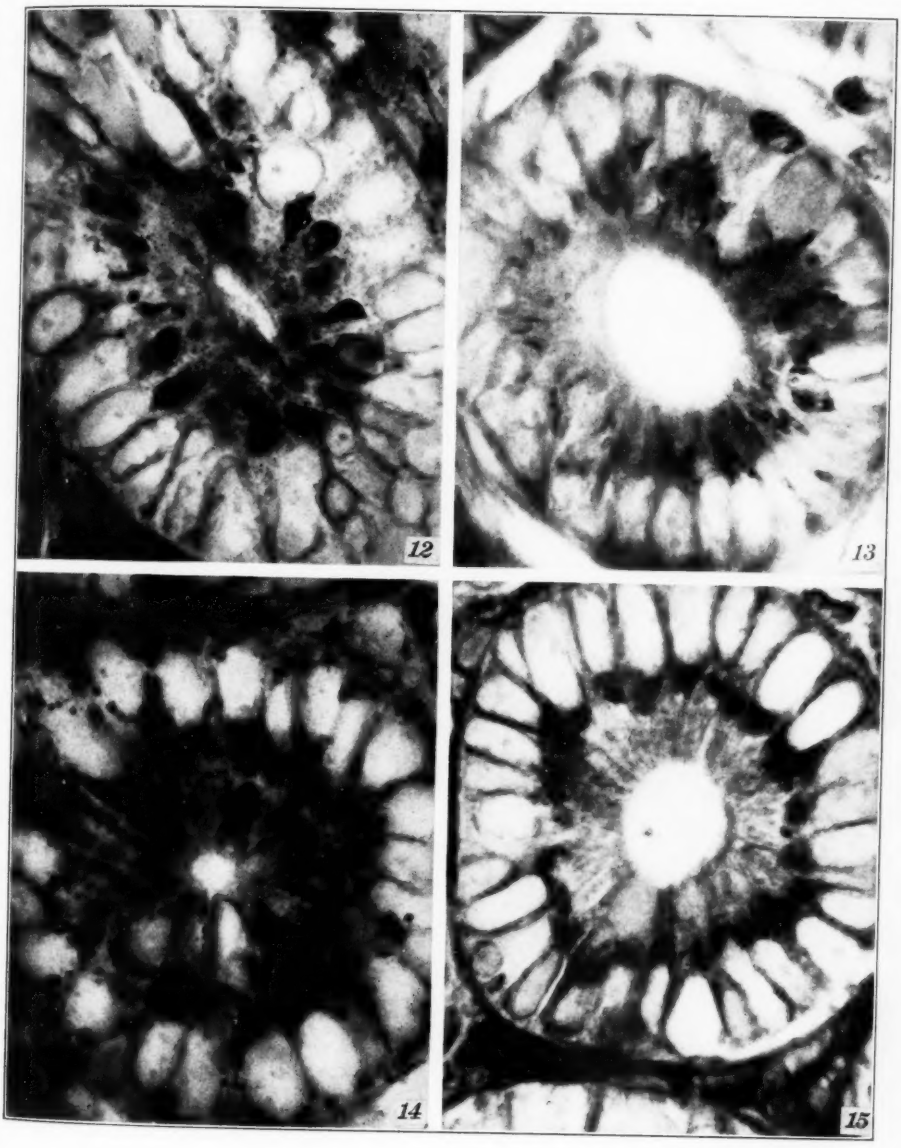
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Plate 75

Figs. 16, 17 and 18. Revealing the structure of the Golgi material in nerve cells of spinal ganglia of normal healthy rodents. Observe the diverse morphology of the apparatus in the larger and smaller cells. In the former the Golgi apparatus is dispersed, forming a broken network throughout the cytosome (Fig. 18). The Golgi substance in the smaller cells forms a compact mass around the nuclear membrane (Fig. 16). This material was treated by Ludford's variation of Nassonov's Golgi technique and stained by orange G in 95 per cent alcohol.

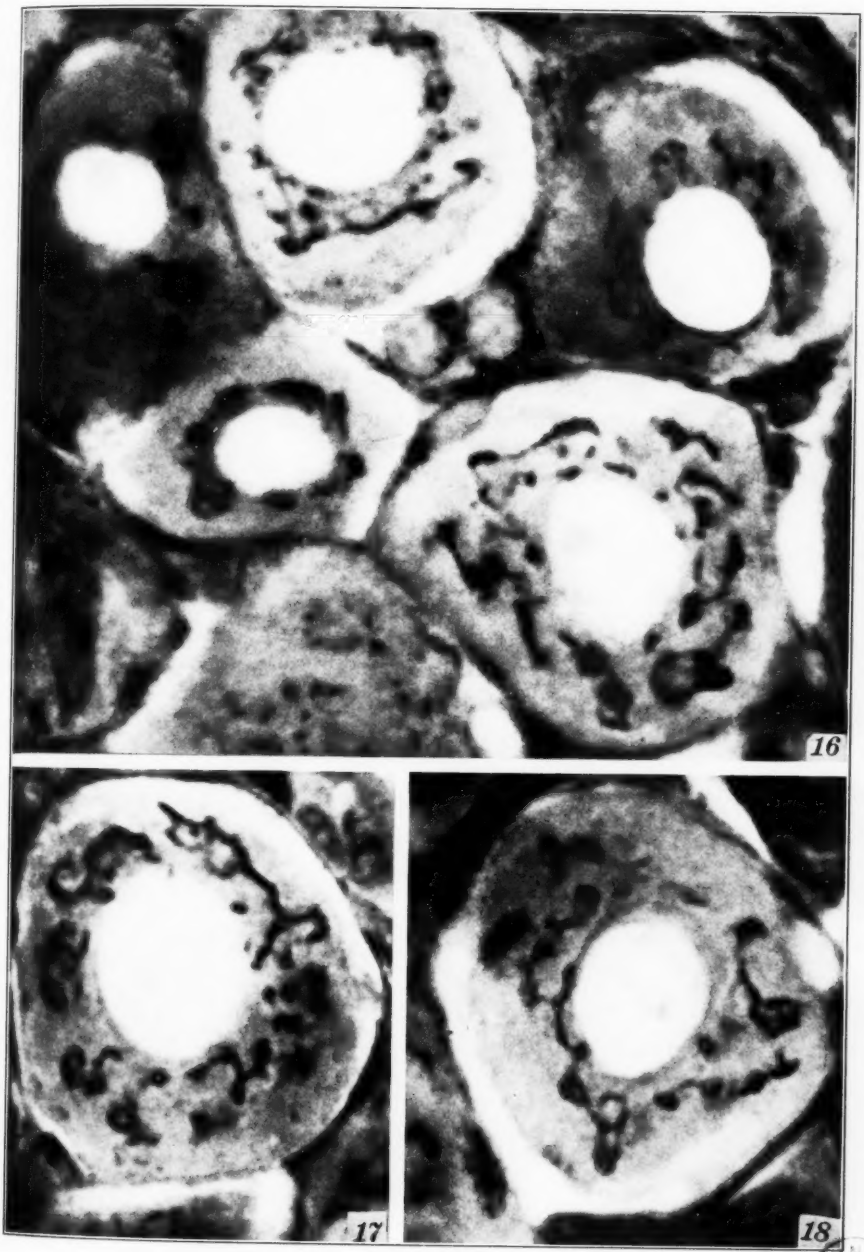
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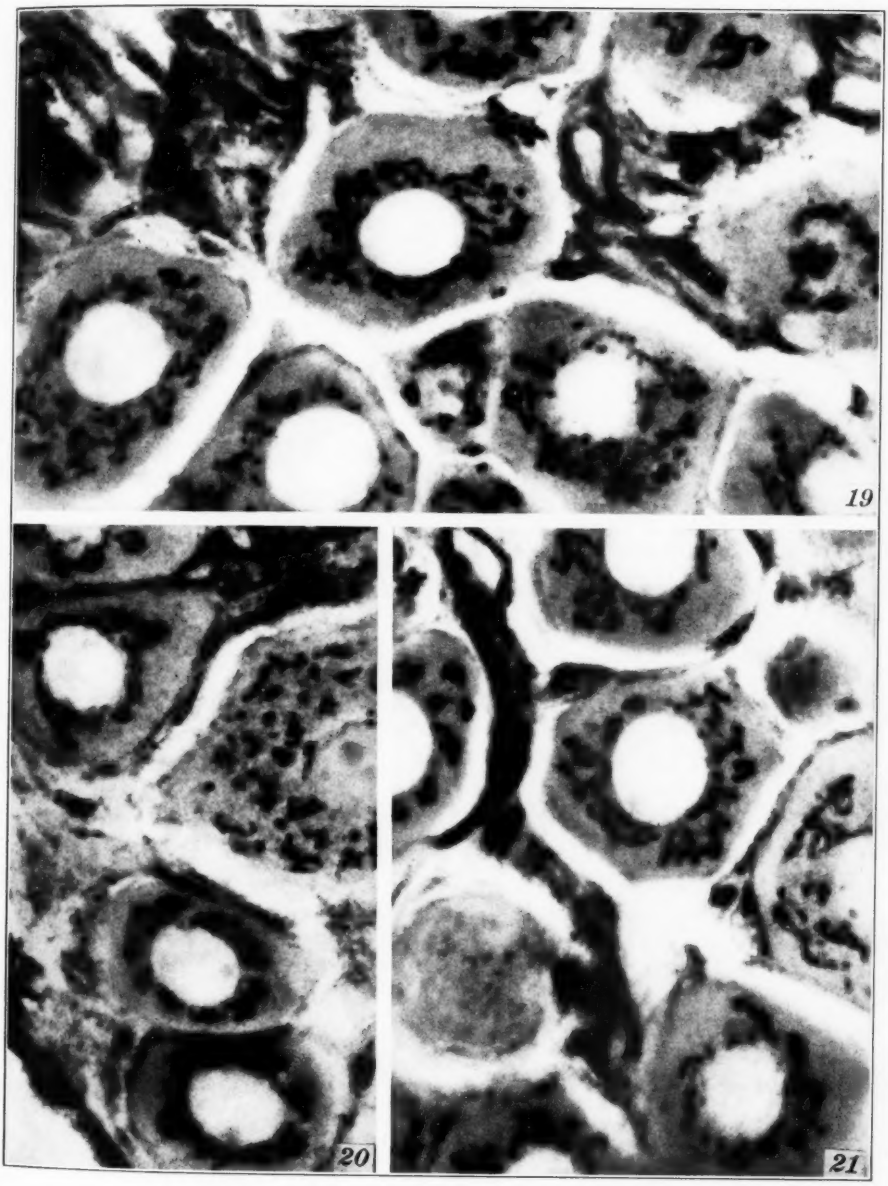
Plate 76

Figs. 19, 20 and 21. Depicting nerve cells of spinal ganglia in a rat following massive injection of morphine hydrochloride, obtained at intervals from 3 to 5 hours after administration of the drug. Fig. 19 shows the condition of the Golgi apparatus 3 hours after injection. In both larger and smaller cells the Golgi material has undergone a slight fragmentation and hypertrophy, which is apparent when compared with the appearance of the apparatus in normal material (see Figs. 16, 17 and 18). Figs. 20 and 21 represent material taken 5 hours after a like dose in which the Golgi apparatus presents a similar appearance to that seen in Fig. 19. In Fig. 20 the apparatus in the smaller cells has undergone less fragmentation than in the larger, but displays a reaction to the drug in the form of a slight hypertrophy.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HISTOLOGY OF THE CORONARY ARTERIES AND THEIR BRANCHES IN THE HUMAN HEART *

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The descriptive literature on the coronary arteries and their branches is remarkable for its paucity. This is all the more extraordinary in view of the fact that diseases of the coronary arteries are occupying more attention today than ever before. With the exceptions of the reports by Wolkoff,[1] and Ehrich, de la Chapelle and Cohn,[2] few significant contributions have been made to this field during the last 15 years. This fact, together with a need for an accurate description of the age period changes in the finer myocardial vascular channels, has led us to report our investigations in this field. It seemed worth while to make a systematic histological study of the main coronary vessels and myocardial twigs, ranging in caliber from moderate sized branches down to capillaries, in order to determine their normal structure as represented in the first eight decades of life, and to observe whether or not there were any significant differences in the myocardial vessels in the various parts of the heart. Our observations, in common with others, have shown that the variations in structure of the coronary branches are not inconsiderable, even for the same age period. Since the reported observations are based largely on a study of relatively few normal hearts and because of the difficulty in obtaining material that would give a fair representation of what might be called the normal coronary vessel, these studies were made on fifty carefully chosen specimens. Diseases that were likely to implicate the coronary vessels were excluded, as were those hearts that showed obviously diseased coronary arteries on gross inspection.

METHODS AND MATERIALS

The hearts were fixed in 10 per cent formalin saline † and blocks cut according to the standardized method of Gross, Antopol and Sacks.[3] By this technique

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† Solution of formaldehyde U. S. P. 10 parts, 1 per cent sodium chloride solution 90 parts. This solution is rendered neutral with a weak alkali.

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six blocks are obtained representing those areas of the heart that experience has shown are most likely to present pathological changes. They can reasonably be called strategic areas in the heart. Together with these six blocks there was also removed one block from the left circumflex coronary artery, 1 cm. from its ostium; one from the left anterior descending branch, 2 cm. from the ostium of the left circumflex coronary artery; one from the right circumflex coronary artery, 1 cm. from its ostium; and one from the posterior descending branch, 0.5 cm. below the auriculoventricular sulcus. The blocks were dehydrated and infiltrated in the usual manner. In the later age periods it was occasionally necessary to decalcify the specimen. Seven and one-half micron sections were cut from each block and stained as a routine with hematoxylin and eosin and Weigert's elastic and Van Gieson's connective tissue stains. Since we were primarily interested in tissue changes rather than infiltrations, relatively few fat stains were done.

General Considerations

In order that the nature of our material may be understood more clearly it is necessary to define our conception of what, for purposes of this report, constitutes a normal vessel. As is well known, certain progressive changes, particularly affecting the intima of the coronary arteries, appear soon after birth. These changes consist of splitting of the lamella elastica interna, the appearance of muscular elements between these split layers (the so-called musculo-elastic layer *) and the further development of elastic and connective tissue elements internally to the latter. It is the opinion of several authors that some of these changes (development of elastic-hyperplastic and connective tissue layers) already constitute early stages of sclerosis, particularly since fatty changes not infrequently occur concomitantly with them. Whether these uniformly occurring phenomena should be placed under the category of pathological processes becomes a matter of definition.

In order to avoid confusion and to simplify the problem we shall not attempt to make this differentiation, since it would lead us into the rather philosophical question as to whether or not the occurrence of pathological changes can be considered a normal process, and to the more theoretical question as to what constitutes a pathological change. It will suffice us to consider the development of these internal layers as normal and to exclude as abnormal the deposition and formation in them in appreciable amounts of such addi-

* We prefer the term "musculo-elastic layer" to "elastic-muscular layer."

tional elements as calcium salts, lipoid crystals, blood vessels and cells of inflammation.

While such a classification as to the normality of a blood vessel is arbitrary, it has the advantage that one must select as a base line such material as shows the least departure from the simple structure represented in the newborn. It also obviates the necessity of including such conspicuous changes as can legitimately be considered under the category of atherosclerosis. Limiting our material in this manner makes it increasingly difficult to find normal vessels in the later age periods. However, with careful selection cases can be found that show a minimum of such changes, even in quite advanced age periods.

In order to define more accurately the problems to which particular consideration was given in these studies it might be well to give first a brief description of the main coronary artery structure which represents a cross-section of opinions gathered from the works of Colucci,[4] Edholm,[5] Faber,[6] Jores,[7] Wolkoff,[1] Bork,[8] Ehrich, de la Chapelle and Cohn,[2] and Spalteholz and Hochrein.[9] This will be followed by a brief description of the small myocardial twigs, based on Wolkoff's description.

According to present conceptions the larger coronary vessel consists of three main layers, adventitia, media and intima. The adventitia is made up of a meshwork of connective tissue whose density increases with age. Particularly in the inner layers of the adventitia are to be found elastic fibers running largely in a circular direction. The media consists of smooth muscle, for the most part circular in arrangement. Scattered among the smooth muscle fibers are circularly arranged elastic elements that are generally more conspicuous toward the outer layers of the media. The intima consists at birth of a single elastic lamella (lamella elastica interna) covered with flat endothelium. With increasing age the intima becomes more complex, this complexity being visible chiefly in the larger vessels and main trunks. The first change consists of splitting of the lamella elastica interna into two membranes between which smooth muscle fibers appear, running at times diagonally but generally in a longitudinal direction. This constitutes the musculoelastic layer. The outermost of these elastic membranes continues to represent the border line between intima and media and accordingly retains the name lamella elastica interna. The innermost

layer, as will be seen, undergoes numerous changes in addition and is referred to as the "inner limiting membrane." Because of the confusion of this term with "lamella elastica interna" we prefer to designate it "secondary intimal elastic membrane." Fine longitudinal elastic fibers are found between the smooth muscle cells of the musculo-elastic layer. Already in the earlier age periods breaks in continuity begin to appear in the lamella elastica interna. Through these breaks smooth muscle cells from the media may be seen pressing themselves into the musculo-elastic layer. On the other hand, by a process of splitting of the secondary intimal elastic membrane, the elastic-hyperplastic layer is formed. This layer takes on great proportions with developing age. Finally, the inner portions of this layer develop a preponderance of connective tissue elements (collagen) and form the so-called connective tissue layer. These last three mentioned subdivisions of the intima increase in thickness with age until the intima eventually becomes considerably thicker than the media. Simultaneously, however, with the development of these layers, areas of discontinuity appear within them so that at times it is difficult to distinguish their limiting borders. In places, one or another of these layers may be missing. In later age periods calcific and atherosclerotic changes occur in the intima.

According to Wolkoff the adventitia of the small myocardial twigs consists of a wide connective tissue feltwork intermingled with elastic tissue which is more concentrated toward the media but forms no definite external elastic lamella. The media consists of circular smooth muscle and elastic fibers. The intima is made up of flat endothelium resting on a lamella elastica interna consisting of anastomosing longitudinal bands of elastic tissue. Wolkoff observed no age period changes in these vessels.

In analyzing our material an attempt will be made to follow the evolution of each main layer from birth to the eighth decade, giving thus a dynamic picture of the changes that take place. Since our observations indicate that by no means do the age period changes in some of the smaller vessels occur, so to speak, at the same tempo in the various parts of the myocardium, an attempt will be made to point out these differences. It will be seen that to discuss the extent of progressive changes in the so-called myocardial arteries is of relatively little value unless indication is given as to the location of the vessel in the heart.

It is to be borne in mind that the description must of necessity be one representing the average of a number of preparations, that very wide variations occur in the different parts of the same vessel, and that a given change is seldom uniform even throughout one microscopic section. In particular, the progressive changes in the intima are unevenly distributed so that it is thrown up into irregular plateaus, and our discussion will represent the average process taking place in a given section.

Because of the wide variations in individual vessels much of the published matter loses considerable significance as the individual variations cannot be considered along statistical lines. A notable exception to this is the report of Ehrich, de la Chapelle and Cohn.[2] In our studies an attempt was made to examine considerable material in the very early age periods, as this has received scant attention. Our description, therefore, will be based on the sequence of events as they are found month by month in the earliest age periods and within very close periods of time thereafter until the end of the first decade. An interesting chapter in the discussion of coronary artery structure, to which apparently little or no attention has been paid in the past, will be the description of the histology of the myocardial twigs, arterioles and capillaries.

Left Circumflex Coronary Artery

Intima: In the first two months of life the intima of this vessel is confined practically to a single layer of flat endothelium which rests on a single continuous lamella elastica interna. As early as the third month this elastic membrane may begin to show areas in which splitting has taken place. These split areas enclose for the most part longitudinal fibers of smooth muscle (Fig. 1).

From this time on the splits become more extensive and conspicuous, so that by the end of the first year one not infrequently encounters specimens in which the entire circumference of the vessel shows a complete doubling of the lamella elastica interna. In such cases the outermost elastic layer (the lamella elastica interna proper) is generally thick and more or less continuous on cross-section, the innermost (secondary intimal elastic membrane) is often more delicate, may be discontinuous and shows many of its elastic fibers running in a longitudinal direction. The smooth muscle between these layers

increases in bulk and often shows delicate longitudinal elastic fibers running between them (the musculo-elastic layer) (Fig. 2).

Simultaneously one also begins to encounter a series of progressive changes in the intima which can be stated briefly as represented by the appearance of discontinuities in the lamella elastica interna (already seen in the third month) and continued splitting of the secondary intimal elastic membrane (generally longitudinal elastic fibers) with the formation of connective tissue between the elastic fibers constituting the so-called elastic-hyperplastic layer (end of the first year).

Wherever discontinuities appear in the lamella elastica interna the musculo-elastic layer lies in intimate relation to the subjacent media. It will be shown later that in such underlying layers of the media the smooth muscle cells may also run in a longitudinal direction, with the result that it becomes impossible sharply to delimit media from intima (Figs. 3 and 6). This will be termed "border disappearance" and the longitudinal smooth muscle layer occupying these areas will be referred to as the "intermediary layer." The frequency with which this intermediary layer is found will be taken up under the description of the media.

By the end of the second year the development of the elastic-hyperplastic layer has become quite conspicuous. The intima at this time, however, with few exceptions, still remains considerably narrower than the media. The above described changes, namely, proliferation of the elastic-hyperplastic layer, growth of the musculo-elastic layer and appearance of discontinuities in the lamella elastica interna, with development of the intermediary layer, continue progressively and are quite well established by the end of the first decade.

During the second decade the musculo-elastic layer becomes prominent and border disappearance is quite regularly met with. With the development of the elastic-hyperplastic layer even in the early age periods (end of the first year) the simple schema of structure for the musculo-elastic layer becomes altered in several respects. First, the smooth muscle elements are no longer confined between two elastic membranes but can be seen spreading irregularly so that they are found encroaching on the elastic-hyperplastic layer. They also occur irregularly distributed throughout the circumference of the intima, appearing in greater concentration in some areas and in

lesser concentration in others. It is, therefore, generally difficult to speak of a sharply defined musculo-elastic layer after the first five years of life.

During the second decade the width of the intima generally equals from one-half to three-quarters that of the media, although in places it may exceed the latter. One also encounters occasionally a transformation of the elastic-hyperplastic layer into dense collagenous tissue, thereby forming the connective tissue layer. This transformation is met with somewhat more frequently during the third decade but does not become a regular constituent of the intima until the fourth decade. Meanwhile, during the third decade the intima not infrequently equals or exceeds the media in width.

From this time on the intima continues to widen, so that by the end of the fifth and the beginning of the sixth decade it is generally several times the thickness of the media. With the appearance of consistently well marked connective tissue layers one not infrequently encounters the deposition of calcium salts and lipoid crystals in the latter. Quite apart from this, however, from the fifth decade on one begins to note encroachment of the thickened intima on the media. In such cases one may see a decrease in the muscular elements of the musculo-elastic layer. These changes occur in exaggerated form during the sixth, seventh and eighth decades (Fig. 4).

Media: The media of the left circumflex coronary artery in its simplest form consists of a band of circularly arranged smooth muscle intermingled with elastic fibers which run for the most part also in a circular direction. These elastic fibers are irregular in their distribution. They occasionally appear in greater concentration toward the inner half of the media but are usually more conspicuous toward the outer half (Fig. 5). The quantity of elastic fibers in the media seems to increase somewhat with age. In those areas of the media that lie immediately beneath a thickened portion of the intima, especially if the latter encroaches on it, the elastic fibers are generally more prominent.

In the second month of life one begins to note bundles of longitudinal smooth muscle in the media which are generally situated immediately beneath the intima (Fig. 5), but are occasionally seen lying more deeply. In some areas these longitudinal smooth muscle bundles, interspersed with longitudinal elastic fibers, may become so extensive as to encircle the entire circumference of the vessel. In

such cases, however, there is almost invariably seen border disappearance, so that the longitudinal smooth muscle becomes a component of the intermediary layer (Figs. 3 and 6).

Toward the end of the first year irregular areas of longitudinal smooth muscle begin to appear somewhat more frequently. They occur with great regularity as a component of the intermediary layer during the second decade.

During the second year of life one begins to observe the presence of collagenous fibers in the media. These, however, do not increase conspicuously in quantity until the second decade. They become very prominent from the middle of the fourth decade on.

When sclerotic changes begin to appear in the intima there are seen frequently atrophic changes in the muscular elements of the media, with the encroaching elastic-hyperplastic intima dipping deeply into it. Vasa vasorum are not found normally in the media. With advancing atherosclerotic transformation, however, blood vessels may appear in the media and penetrate into the sclerotic intima.

Adventitia: This outermost supporting structure of the blood vessel consists at birth of relatively loose bundles of connective tissue running circularly and diagonally, interspersed with circular and longitudinal elastic fibers. The latter are so decidedly concentrated at the media-adventitia border that they may be considered to form a lamella elastica externa, even though this is not a continuous membrane. The thickness and quantity of these elastic fibers increase rapidly during the first year of life. The increase thereafter until the end of the first decade is proportionate to the growth of the vessel. From then on the fibers continue to concentrate immediately outside the media-adventitia border, but decrease in quantity throughout the rest of the adventitia.

The connective tissue fibers become progressively more compact from birth to the end of the eighth decade.

Review of Characteristic Histological Features of the Left Circumflex Coronary Artery

In reviewing these findings in the left circumflex coronary artery certain points may be emphasized as follows:

1. The early splitting of the lamella elastica interna.

2. The rapid formation of the elastic-hyperplastic layer.
3. The diffuse and irregular growth of the musculo-elastic layer, which soon passes internally beyond the border of the so-called secondary intimal elastic membrane.
4. The rapid loss in continuity of the secondary intimal elastic membrane.
5. Existence of longitudinal smooth muscle in the media.
6. The formation of the intermediary layer by fusion of the musculo-elastic layer with smooth muscle of the media. This occurs particularly after the first decade.
7. The rapid concentration of elastic fibers in the adventitia in the zone immediately external to the media, with the disappearance of these fibers from the rest of the adventitia.

A comparison of the development of these structures, as between the various coronary arteries, will be discussed following the description of each vessel.

Left Anterior Descending Branch

Intima: This vessel generally differs from the other coronary branches in that its intima appears to be considerably advanced in progressive changes over that of the left circumflex coronary artery. At birth one can see irregular elastic-hyperplastic thickenings of the intima, fine splitting of the lamella elastica interna and early formation of the musculo-elastic layer. By the second month of life discontinuities are noticed in the lamella elastica interna with here and there the formation of the intermediary layer in areas of border disappearance. Toward the end of the first year of life the intima may equal the width of the media in places. This is not found with any degree of regularity, however, until the end of the first decade. By the fifteenth year border disappearance may have become so complete that the intermediary layer occasionally occupies the entire circumference of the vessel. By the end of the second decade the intima is often twice the thickness of the media. This thickening, largely due to the growth of the elastic-hyperplastic layer, continues rapidly so that by the fourth decade it is frequently several times the width of the media.

A somewhat less obvious difference in the development of the intima in the left anterior descending branch, as compared with that

of the left circumflex coronary artery, is the appearance of the connective tissue layer somewhat earlier, namely, during the end of the third decade, although its regular appearance is not encountered until the fourth decade.

The orderly arrangement of the musculo-elastic layer, which is already well established during the middle of the first year, is lost much earlier in the left anterior descending branch than in the other vessels. From the second year of life on one frequently encounters the musculo-elastic layer so advanced in development that it spreads within the elastic-hyperplastic zone.

One of the most important characteristics of the left anterior descending branch is the appearance of lipoid and calcific deposits more frequently, more conspicuously and earlier in this vessel than in the other coronary branches. As a consequence encroachment of the intima on the media is met with very frequently from the fourth decade on. After the fifth decade the intima is rarely encountered without lipoid and calcific deposits.

Media: The media of the left anterior descending branch shows no conspicuous differences from that of the left circumflex coronary artery until the latter half of the second year of life when the more frequent occurrence of the intermediary layer alters its characteristic structure. As in the left circumflex coronary artery, the growth of the media on the whole parallels that of the myocardium. The elastic fibers which are generally richer in the left anterior descending branch than in the other coronary branches increase slightly in quantity with age until the first decade. From this time on they seem to diminish abruptly in quantity, except in those areas underlying sclerotic plaques, as mentioned in the left circumflex coronary artery description. The distribution of the elastic fibers in this vessel is also generally more toward the outer half of the media. The collagen fiber component of the media can be seen well during the second year of life. Its increase, however, is not rapid. It becomes conspicuous during the third decade.

Adventitia: The structure and development of the adventitia of this vessel is similar to that described for the left circumflex coronary artery. Its width, however, is generally greater than that of the other coronary branches. Decrease in the extent of elastic fibers distributed throughout the adventitia occurs from the end of the first decade on, as found in the left circumflex coronary artery.

Review of Characteristic Histological Features of the Left Anterior Descending Branch

The important points to be emphasized as peculiar to the left anterior descending branch are:

1. Earlier appearance of the elastic-hyperplastic layer.
2. Rapid progress in the development of the elastic-hyperplastic layer with marked thickness of the intima.
3. Earlier appearance and more marked deposits of lipoid crystals and calcium salts in the intima.
4. More rapid development and spread of the musculo-elastic layer.
5. Earlier and more frequent occurrence of the intermediary layer.
6. Somewhat greater width of the adventitia.

Right Circumflex Coronary Artery

Intima: On the whole the intima of this vessel maintains its simplicity of structure for a longer period of time than that of the left anterior descending branch or the left circumflex coronary artery. While minute splittings of the lamella elastica interna can at times be detected in the seventh week of life this does not become conspicuous until the latter part of the first year, at which time the musculo-elastic layer also begins to make its appearance. Elastic-hyperplastic changes appear with greater regularity by the end of the eighteenth month and discontinuities of the lamella elastica interna, which are rare up to the end of the second year, also begin to appear more frequently at this time.

The continued growth of the intima differs from that of the left circumflex coronary artery and the left anterior descending branch in several respects. The musculo-elastic layer is generally narrower than in the other vessels. The rapidly and earlier developing connective tissue layer encroaches on the musculo-elastic layer, producing atrophy of its smooth muscle fibers. Connective tissue changes in the elastic-hyperplastic layer can sometimes be seen in the second half of the first decade. They are encountered with considerable regularity during the third decade and thereafter develop more frequently and rapidly than in the other coronary vessels. Indeed,

the connective tissue layer is generally more conspicuous in this vessel during the later decades than is the elastic-hyperplastic layer.

It has been mentioned that the lamella elastica interna discontinuities begin to appear with some degree of frequency after the second year of life. On the whole, however, they are generally less marked in this vessel. This is also true for the incidence of border disappearance.

The intima is generally half as wide as the media during the second decade and equals it during the third decade. During the fourth decade and thereafter, however, it generally becomes very wide because of the considerable development of the connective tissue layer.

Media: The media of the right circumflex coronary artery corresponds in development very closely to that of the left circumflex coronary artery with the following sharp differences. (1) The intermediary layer is encountered with less frequency, as are the longitudinal smooth muscle bundles elsewhere in the media, and (2) the connective tissue elements (collagen fibers) appear much earlier. With respect to the latter it may be said that even as early as toward the latter half of the first decade they are seen with a fair degree of regularity. They rapidly become more conspicuous from the second decade on. The quantity and distribution of elastic fibers in the media are similar to that found in the left circumflex coronary artery.

Adventitia: The adventitia of the right circumflex coronary artery shows in its connective tissue elements a type of development that is similar to the two vessels previously described. Its width is generally similar to that of the left circumflex coronary artery and the elastic fibers behave very much like those of the adventitia of the left circumflex coronary artery in respect to concentration.

Review of Characteristic Histological Features of the Right Circumflex Coronary Artery

The important points to be emphasized as peculiar to the right circumflex coronary artery are:

1. Later appearance of splitting of the lamella elastica interna.
2. Generally narrower width of the musculo-elastic layer and its later atrophy, because of the considerable development of the connective tissue layer in the intima.

3. The later and less marked discontinuities in the lamella elastica interna.
4. Earlier and considerable development of the connective tissue layer.
5. The growth in width of the intima as a whole is similar to that of the left circumflex coronary artery.
6. Less frequent occurrence of border disappearance and the intermediary layer.
7. Earlier appearance of collagen fibers in the media.

Posterior Descending Branch

Intima: In describing the histology of the posterior descending branch one must bear in mind the fact that the structure varies considerably, depending on whether the posterior descending branch is large or small. This variation is much greater for the posterior descending branch than for the other coronary vessels. If the vessel is large its structure and age period changes will approximate those described for the right circumflex coronary artery. Generally, however, the vessel is considerably smaller, in which case the intima maintains its simple form for a much longer period of time.

Assuming the smaller form to be the more frequent one may say that occasionally minute splittings of the lamella elastica interna with development of longitudinal smooth muscle fibers may be encountered during the first year of life but do not become very marked until the latter half of the first decade. Elastic-hyperplastic changes are very scant until the middle of the second decade, at which time the lamella elastica interna discontinuities also begin to appear with some degree of regularity.

The intima reaches in width half that of the media, generally during the third decade. Toward the end of the fourth decade it equals the media in width but grows rather slowly. Occasionally, during the fourth decade, irregular thickenings many times the width of the media may be found. The connective tissue layer generally does not make its appearance until the fifth decade. In keeping with the slower tempo of changes in the posterior descending branch lamella elastica interna discontinuities are encountered much less frequently, as are border disappearances and the development of the intermediary layer. The latter occurs very irregularly.

It follows from this description that sclerotic changes in the posterior descending branch make their appearance later in life than in the other main vessels.

Media: The media of the posterior descending branch shows the least amount of elastic tissue of the four vessels described. These fibers tend to be more concentrated toward the external half of this layer and generally disappear practically completely after the first decade. On the other hand, the posterior descending branch resembles the right circumflex coronary artery in the earlier appearance of connective tissue elements, which are fairly well discernible toward the end of the first decade and become more conspicuous thereafter.

Adventitia: The adventitia of the posterior descending branch is narrower than in the other vessels described. At times, however, it may be considerably wider than the others. Its elastic fibers are generally fairly sparse, increase somewhat from the second year of life to the end of the fifth year of life, and decrease again thereafter.

Review of Characteristic Histological Features of the Posterior Descending Branch

The important points to be emphasized as peculiar to the posterior descending branch when it occurs as a small vessel are:

1. Very late appearance of splitting of the lamella elastica interna and development of the musculo-elastic layer.
2. Very late occurrence of discontinuities in the lamella elastica interna. These are rather inconspicuous.
3. Very late appearance of elastic-hyperplastic changes.
4. Very late appearance of the connective tissue layer.
5. Very slow increase in the thickness of the intima as a whole.
6. Late appearance of lipoid and calcific deposits.
7. Very infrequent occurrence of border disappearance and intermediary layer.
8. Scant elastic fibers in the media and their early disappearance.
9. Generally narrower adventitia.

Myocardial Vessels

The coronary artery branches within the myocardium will be described under the categories of myocardial arteries, arterioles and capillaries. Each vessel will be considered in respect to age period

changes and location within the myocardium, based on an examination of the standardized blocks. It will be seen that of these three types of vessels the myocardial artery is the only one showing marked differences in structure and evolution dependent on its site within the heart.

Myocardial Arteries: Under this category are considered those vessels that possess three definite coats with a media consisting of more than one layer of circular smooth muscle fibers. Even in the earliest age periods one can already note remarkable differences between the medium sized twigs found in the various parts of the heart. The general arrangement of the component layers of these vessels is as follows. The adventitia is made up of a loose feltwork of connective tissue fibrillae with very sparse and delicate elastic fibers, both elements running in all directions. It may be mentioned here that the adventitial elastic fibers of the myocardial arteries in the auricles tend to be somewhat more numerous than elsewhere in the heart. On cross-section the adventitia is generally oval, filling in the interstices of the myocardial bundles. Because of this the width of the adventitia varies considerably. The media is composed of several layers of circular smooth muscle. The lamella elastica interna is made up of longitudinal elastic fibers. This is covered by flat endothelium.

At birth one occasionally notes circular elastic fibers intermingled with the smooth muscle cells of the media in some of the vessels in the posterior papillary muscle of the left ventricle and in the interventricular septum. These elastic fibers tend to form sheaths around the muscle cells. The continued development of these elastic fibers in the media will be referred to as "elastification of the media." In the fifth year of life one begins to encounter splitting of the lamella elastica interna and elastification of the media somewhat more often in the interventricular septum, but particularly in the posterior papillary muscle of the left ventricle. By the tenth year of life elastification of the media has become even more marked in the posterior papillary muscle of the left ventricle but can also be seen at times in the interventricular septum, left ventricle and pulmonary conus. At the beginning of the third decade the vessels in the posterior papillary muscle of the left ventricle may show elastic-hyperplastic changes of the intima with discontinuities in the lamella elastica interna.

Toward the latter part of the third decade another phenomenon begins to appear, particularly in the posterior papillary muscle of the left ventricle, namely, fusion of the elastic-hyperplastic intima with the elastified media. This is due to the fact that the elastification of the media may have gone on so rapidly that it is no longer possible to distinguish sharply the elastic-hyperplastic changes in the intima from the heavily elastified media. Furthermore, the intima may show smooth muscle fibers within its substance running in a longitudinal direction. At the same time, irregular patches of connective tissue may be found in the media replacing smooth muscle, so that the continuity of the latter is lost (Fig. 7). These changes are found with increasing frequency toward the end of the fourth decade and thereafter in the posterior papillary muscle of the left ventricle.

In the fifth decade splitting of the intima begins to be encountered in the pulmonary conus and auricles. Further progressive changes in these two sites are rather slow to develop. In the auricles especially, they rarely reach stages beyond rather mild media elastification with some intimal elastic splitting. In the seventh and eighth decades many of the vessels lying in the posterior papillary muscle of the left ventricle, interventricular septum and left ventricle have lost so much of their smooth muscle substance, developed so many connective tissue fibers in the media and intima, and undergone so much elastification of both these layers that they have been largely converted into elastic or fibro-elastic tubes. The possible physiological significance of these changes will be discussed later.

Arterioles: The arterioles of the myocardium differ from the arteries in several important respects. Apart from being considerably narrower in diameter, they possess a media made up of only a single layer of smooth muscle, their lamella elastica interna presents a beaded appearance until the eighth decade of life and they may or may not possess an adventitia, depending on their location in the myocardium.

The intima consists of a single layer of flat endothelium resting on a lamella elastica interna. This elastic membrane is only faintly discernible during the first two months of life. During this period it is made up of anastomosing, very delicate longitudinal fibers giving a beaded appearance on cross-section. From the third month until the seventh decade the fibers of the lamella elastica interna undergo

a progressive thickening and fusion but almost invariably maintain their beaded appearance on cross-section. Splitting of this membrane may begin to take place during the sixth decade but seldom becomes marked (Fig. 8). The split areas generally enclose collagen fibers.

The single layer of smooth muscle in the media may occasionally undergo atrophy during the very late decades. The atrophy does not generally involve the entire circumference of the vessel.

Many of the arterioles within the myocardium are devoid of an appreciable adventitia. If an arteriole is situated within the adventitia of an artery or within a somewhat heavier trabeculum of connective tissue it may be considered to possess an adventitial coat of its own. This varies considerably in contour and often consists of an irregularly arranged meshwork of collagen fibers intermingled with scant elastic fibers running in all directions. After the second decade of life the elastic fibers become more discernible and there can often be seen a single delicate elastic membrane surrounding the adventitia and forming a lamella elastica externa. This is at times discernible as early as the end of the first year of life.

The only point of interest to note with respect to differences in arterioles according to their site within the heart is that the adventitia is apt to be larger, and richer in elastic fibers in the auricles.

Capillaries: At birth the capillaries consist of delicate tubes made up of flat endothelium resting on a hyaline basement membrane. By the fifteenth year of life the capillaries throughout the heart may show a delicate elastic lamella outside the basement membrane. This, however, does not become conspicuous until the beginning of the sixth decade. There are no conspicuous differences to be noted in capillary structure within the various parts of the heart.

Discussion and Summary

There has been presented in this report a description of the main coronary arteries of the human heart as well as of the myocardial arteries, arterioles and capillaries, as represented in six different areas in the heart (standardized blocks). The description particularly concerns itself with the age period changes that these vessels undergo. It is demonstrated that the main coronary vessels present a succession of progressive changes in the intima, media and adventitia which may be briefly summarized as follows:

1. Splitting of the lamella elastica interna with the formation of the musculo-elastic layer and the secondary intimal elastic membrane.

2. Continued splitting of the secondary intimal elastic membrane to form the elastic-hyperplastic layer.

3. Irregular growth and spread of the musculo-elastic layer into the elastic-hyperplastic zone.

4. Collagenic transformation of the elastic-hyperplastic layer to form the connective tissue layer.

5. Calcific and lipoid deposits in the intima with pressure atrophy of the underlying media and frequent development in the latter areas of additional elastic fibers.

6. Participation of the media and intima in the formation of the musculo-elastic zones termed "intermediary layers."

7. Development of intermediary layers concomitantly with the appearance of discontinuities in the lamella elastica interna, a process termed "border disappearance."

8. Increase in elastic elements in the media during the early age periods with decrease thereafter, particularly in the posterior descending branch.

9. Occurrence of longitudinal smooth muscle bundles in the media; seen with greater frequency during the later age periods.

10. Development of collagen fibers in the media.

11. Condensation of elastic fibers in the adventitia to form a more or less recognizable lamella elastica externa with partial disappearance of these elastic fibers from the rest of the layer with age.

It is further demonstrated that the left anterior descending branch presents these changes (with the few noted exceptions) more frequently and earlier than the other main coronary vessels. The left circumflex coronary artery is next in order chronologically and in the frequency with which these changes occur. The right circumflex coronary artery follows closely the development of the left circumflex coronary artery but possesses peculiarities of its own. The posterior descending branch, especially when it occurs as a narrow calibered vessel, is the last to show the above-mentioned progressive changes. Variations in the tempo with which these phenomena occur are great and even after making a comparison of the several vessels with a knowledge of the age of the individual it may be difficult or, at times, impossible to distinguish definitely the

left circumflex coronary artery, left anterior descending branch and the right circumflex coronary artery from one another. The left anterior descending branch generally may be recognized, however, by its more advanced progressive changes, the posterior descending branch by its markedly retarded developmental changes.

Obviously, the main coronary arteries thicken and stiffen with age. It would seem that vasodilatory changes can occur under these circumstances with increasing difficulty. Whether the rapidly developing intermediary layer may serve as a compensatory factor, *e.g.*, increase the diameter of the vessel by contraction of its longitudinal smooth muscle elements, is a matter for conjecture.

The elastic-hyperplastic changes can safely be considered to show all gradations into the definitely atherosclerotic process.

In the description of the age period changes of the myocardial arteries it has been demonstrated that a curious transformation takes place with age, *i.e.*, elastification of the media, elastic-hyperplastic changes in the intima, fusion of these two layers, atrophy of the smooth muscle elements and development of irregular patches of connective tissue — in short, a fibro-elastic metamorphosis of these vessels. Of greater importance is the fact that this process occurs at different times in various parts of the myocardium. Thus, of the standardized blocks examined, these changes are found first and most frequently in the posterior papillary muscle of the left ventricle; next in the interventricular septum, left ventricle and pulmonary conus, in this order; last and least in the auricles. That such fibro-elastic transformation of these vessels renders them more or less passive, *i.e.*, less susceptible to vasomotor control, seems evident. Narrowing of the larger vessels leading to these areas assumes, therefore, a serious portent, inasmuch as the fibro-elastified vessels probably cannot undergo sufficient vasodilatory changes to compensate for the narrowing. The importance of such vasodilatory changes was emphasized by Smith[10] in 1921. He was interested in the question as to whether or not vasodilatation can occur in the coronary arteries and their branches following the administration of nitrites. His experiments consisted of tying off the distal branches of the left circumflex coronary artery in dogs. As a result of this procedure cyanosis of various degrees was produced in the myocardium, indicative of impending infarction. Following the administration of nitrites he observed, in some of the dogs, dis-

appearance of the cyanosis. In other experiments he measured the blood flow from the same vessels before and after the administration of sodium nitrite. Again, he was able to show a vasodilatory effect in some dogs. Smith concluded that in some dogs there is a communication with adjacent vessels which dilate under the action of these drugs.

If there is a similar vasodilatory compensatory mechanism in the human coronary arteries, as seems likely from observations to be published later, it helps to throw further light on certain observations made by Gross. In 1921 he demonstrated the gradual progressive development with age of wide anastomotic channels, particularly in the septum. Commenting on this observation Marvin [11] disagreed with the fact and suggested that the allegedly greater elasticity of the younger vessels either prevented the injection medium, used by Gross for study of these vessels, from entering the vessels, or squeezed it out into the larger ones.

If the elasticity of the vessel in any way parallels its content of elastic tissue our observations are diametrically opposed to Marvin's suggestion — certainly for the first three decades of life before appreciable fibrotic changes have developed. On the contrary, their very elasticity (tonic) and passivity could serve as a stimulus for the development of compensatory dilatation in other vessels supplying the same areas. In this connection it is of interest to note that these fibro-elastic transformations occur most frequently and most markedly in those areas of the heart that are the most frequent sites of infarction.

Finally, it is demonstrated that the myocardial arterioles present the above-mentioned changes in far less marked form and that the capillaries present with age only irregularly the development of an elastic layer outside its basement membrane.

It is hoped that these findings may serve as a baseline for comparative studies on the vessels of the human heart in disease.

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DESCRIPTION OF PLATES

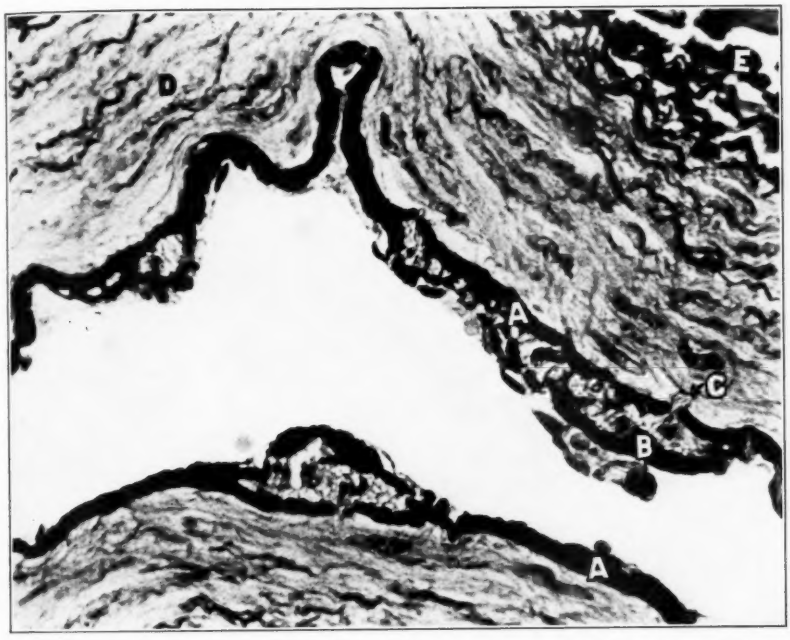
PLATE 77

FIG. 1. Left circumflex coronary artery. Age 3 months. High power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

A = lamella elastica interna; B = secondary intimal elastic membrane; C = smooth muscle cell squeezing its way into intima from media through a discontinuity in the lamella elastica interna; D = media; E = adventitia.

FIG. 2. Left circumflex coronary artery. Age 5 years. High power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

A = lamella elastica interna, more or less continuous and heavy; B = secondary intimal elastic membrane, discontinuous, more delicate; C = musculo-elastic layer; D = elastic-hyperplastic layer; E = discontinuity in lamella elastica interna occupied by smooth muscle cell.



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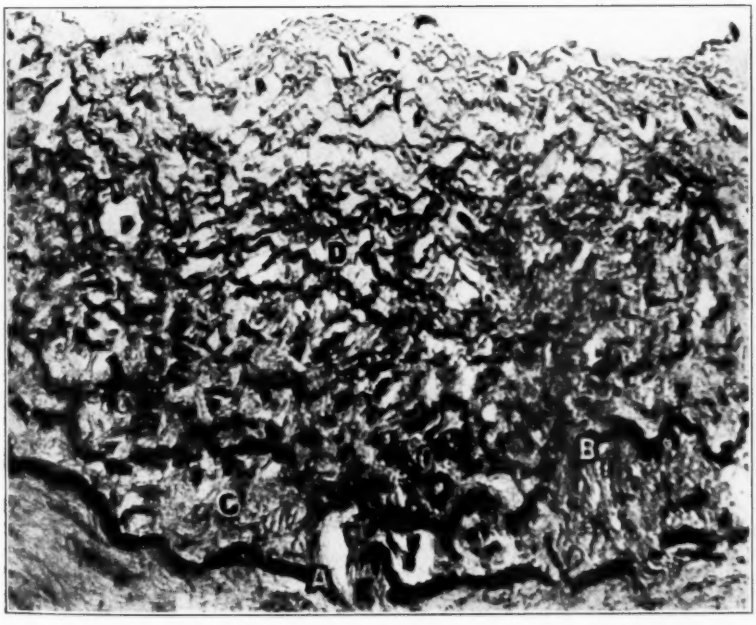

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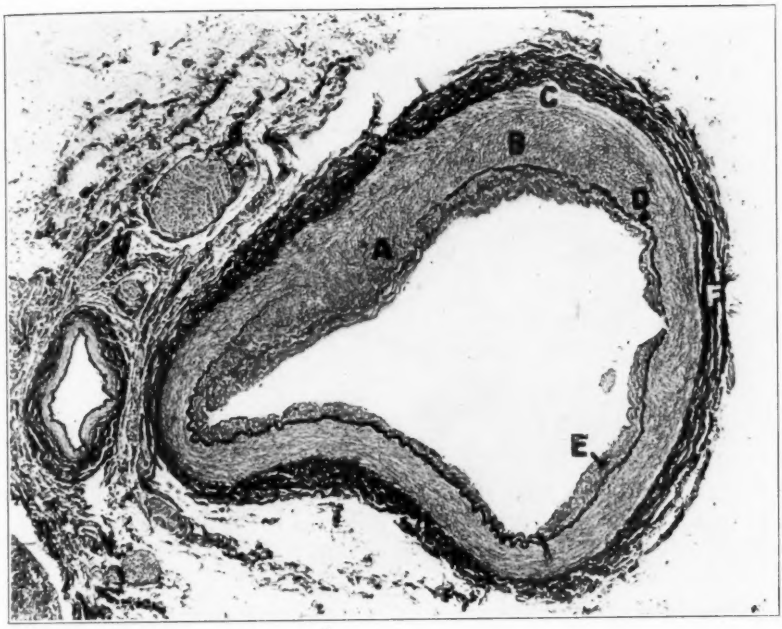
PLATE 78

FIG. 3. Left circumflex coronary artery. Age 18 months. Low power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

A = area of border disappearance occupied by intermediary layer; B = longitudinal smooth muscle in media; C = circular smooth muscle of media; D = lamella elastica interna; E = intima; F = adventitia.

FIG. 4. Left circumflex coronary artery. Age 74 years. Low power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

A = connective tissue layer in intima; B = media; C = atrophic portion of media heavily elastified; D = adventitia; E = lipoid and calcific changes in intima.



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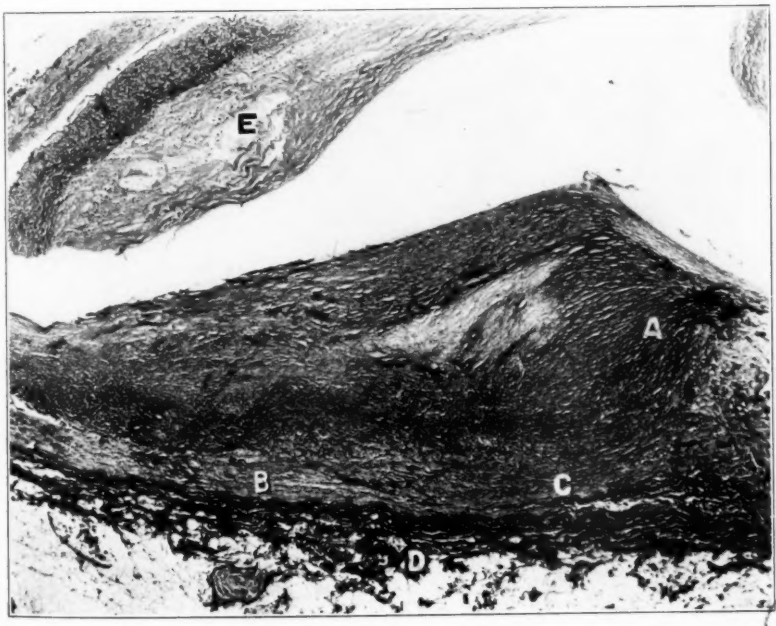

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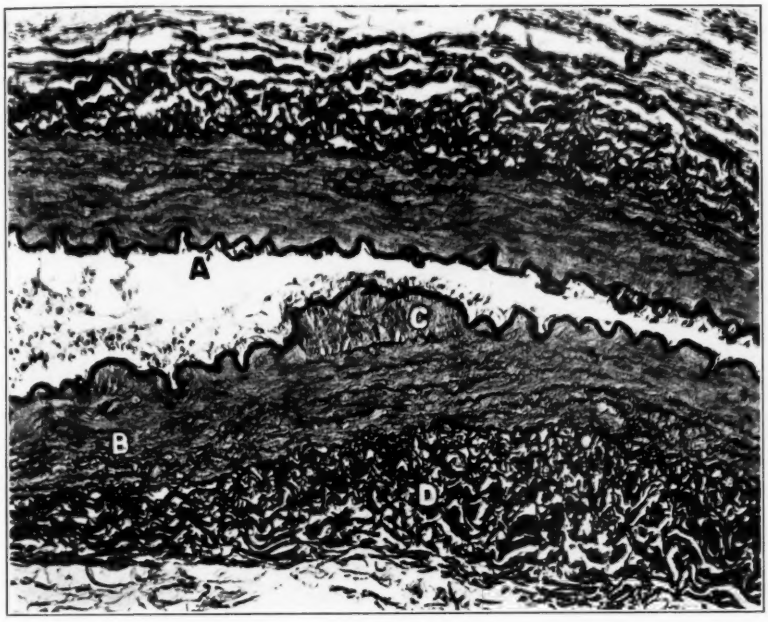
PLATE 79

FIG. 5. Left circumflex coronary artery. Age 2 months. Medium power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain. A = lamella elastica interna; B = media; C = longitudinal smooth muscle bundle in media; D = adventitia.

FIG. 6. Left circumflex coronary artery. Age 18 months. Medium power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain. A = intermediary layer of elastic and longitudinal smooth muscle bundles. Note absence of lamella elastica interna; B = media; C = adventitia; D = intima.



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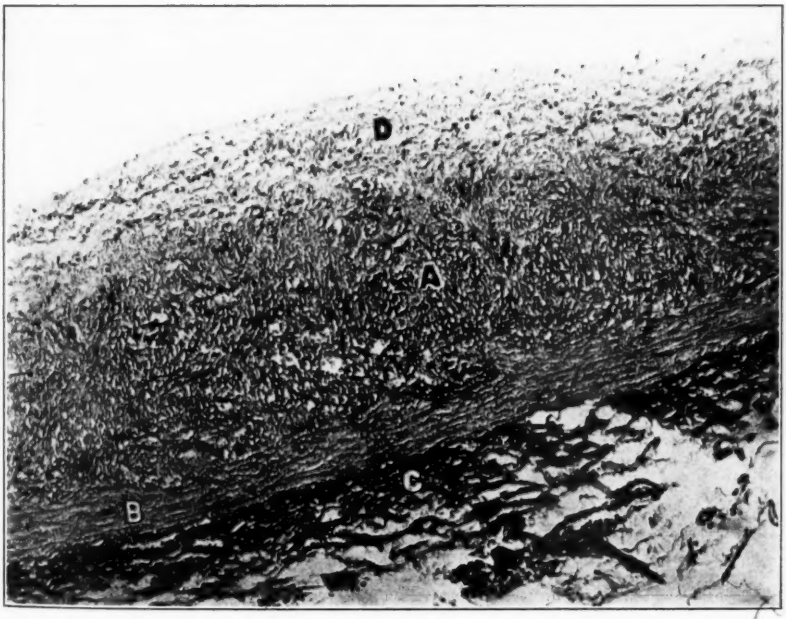

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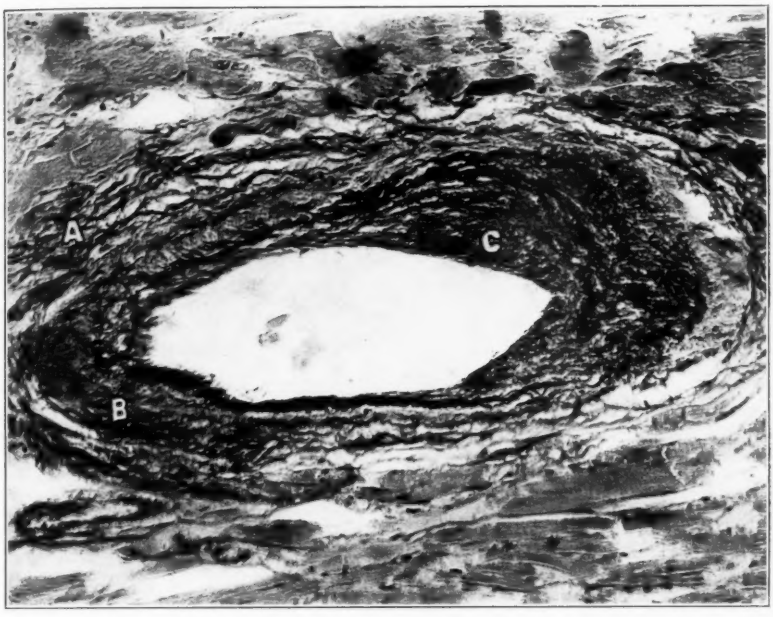
PLATE 80

FIG. 7. Myocardial artery in left posterior papillary muscle showing typical fibro-elastic transformation. Age 40 years. High power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

A = adventitia; B = media showing almost complete loss of smooth muscle with elastic and fibrotic changes; C = elastic-hyperplastic and fibrotic intima.

FIG. 8. Arteriole in the posterior papillary muscle of the left ventricle showing advanced intimal changes. Age 43 years. High power. Cross-section. Weigert's elastic and Van Gieson's connective tissue stain.

A = thickening and splitting of the lamella elastica interna; B = media; C = adventitia.



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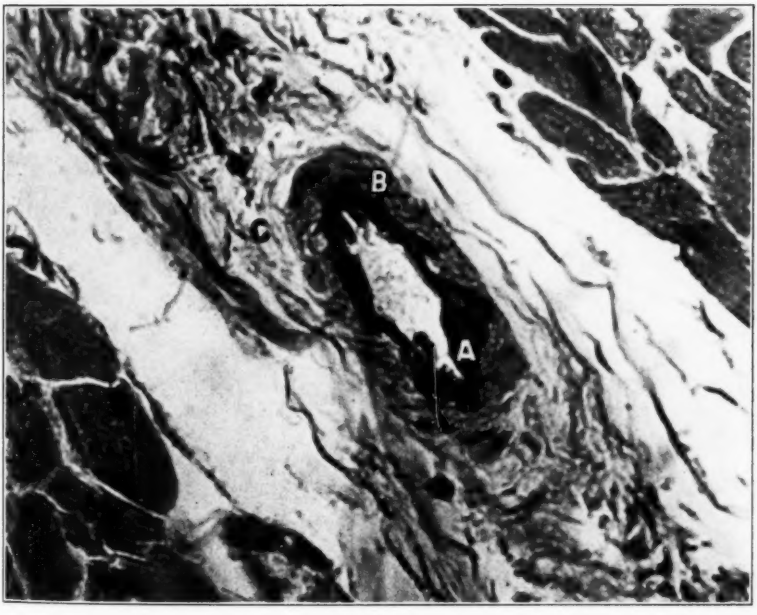

8

EXPERIMENTAL STUDIES ON VENEREAL SARCOMA OF THE DOG *

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Venereal sarcoma has been the subject of considerable experimental work following the observations of Nowinski [1] (1876) and Wehr [2] (1888) that it is readily transmitted from diseased to healthy dogs.

This tumor occurs as single or multiple, nodular or papillary growths commonly on the corona penis or in the vaginal mucosa. It occasionally spreads by metastasis to the nearby lymph nodes but seldom to internal organs. It is readily transmitted by sexual contact, by rubbing the tumor into scarified mucous membrane, or by injecting pieces of the tumor into the subcutaneous tissue. In most instances the tumor remains localized to the genital organs and often regresses spontaneously.

The literature on studies of venereal sarcoma has been reviewed recently by Opie [3] and Feldman.[4] The tumor has been described in Russia, the United States, France, Germany and England (Opie [3]), but there is no accurate information concerning its incidence. During the past 3 years 5 instances of spontaneous venereal sarcoma were observed at the University of Pennsylvania Veterinary Hospital among approximately 30,000 dogs presented for examination. This tumor was much more frequently encountered 10 to 20 years ago, according to Dr. A. Glass and Dr. W. J. Lentz of the veterinary school. It is possible that caretakers of kennels are aware of the ease with which this tumor is transmitted and destroy or segregate affected dogs without consulting a veterinarian.

The nature of this tumor is unknown. According to most investigators it is a very readily transmissible neoplasm, indistinguishable from other mammalian tumors. It was first described

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as a carcinoma but subsequent investigators considered it a round-cell sarcoma. It is often described as a lymphosarcoma, although there is no evidence that the cells forming this tumor are lymphocytes. According to a few workers it is due to a stimulation of tissue cells of the host by a microörganism, perhaps a filterable agent, and is regarded by them as a granuloma.

Gross and Microscopic Characteristics of Venereal Sarcoma

Venereal sarcoma is grayish white, very firm and, unlike lymphosarcoma, cuts with difficulty and does not emulsify when cut up in Locke's solution.

Microscopically (Figs. 7 to 12) it is made up of large round cells subject to little variation in appearance. They are somewhat larger than lymphoblasts, contain a large vesicular nucleus with usually a single intensely basophilic nucleolus, and have abundant cytoplasm, slightly eosinophilic (pale in hematoxylin-eosin preparations). The cells are in contact with each other like epithelial cells, but when detached they resemble large lymphocytes. Differentiation or maturation was never seen in any direction and for this reason the nature of this tumor remains obscure. Small accumulations of lymphocytes occur in older tumors; it is apparent, however, that the tumor is invaded by the lymphocytes and that the tumor cells do not mature into small lymphocytes. Connective tissue is scant in young tumors (Fig. 7); in older tumors it sometimes becomes abundant, separating the sarcoma cells into small nests, as shown in Figure 8. Sections stained with Heidenhain's modification of Mallory's anilin blue stain (Fig. 11), and with Foot's silver stain (Fig. 12), show scant collagenous and reticulum fibers among the large round cells.

Experimental

Two cases of venereal sarcoma were observed by us in December 1929, and each was successfully transmitted to healthy dogs. The first occurred as a nodular growth about 1 cm. in diameter, in the vaginal mucosa of an English bulldog. Parts of the tumor were removed and injected into the groin of 2 dogs and into the peritoneal cavity of a 3rd dog, and rubbed into the scarified penis of a 4th dog. Following biopsy this apparently spontaneous tumor

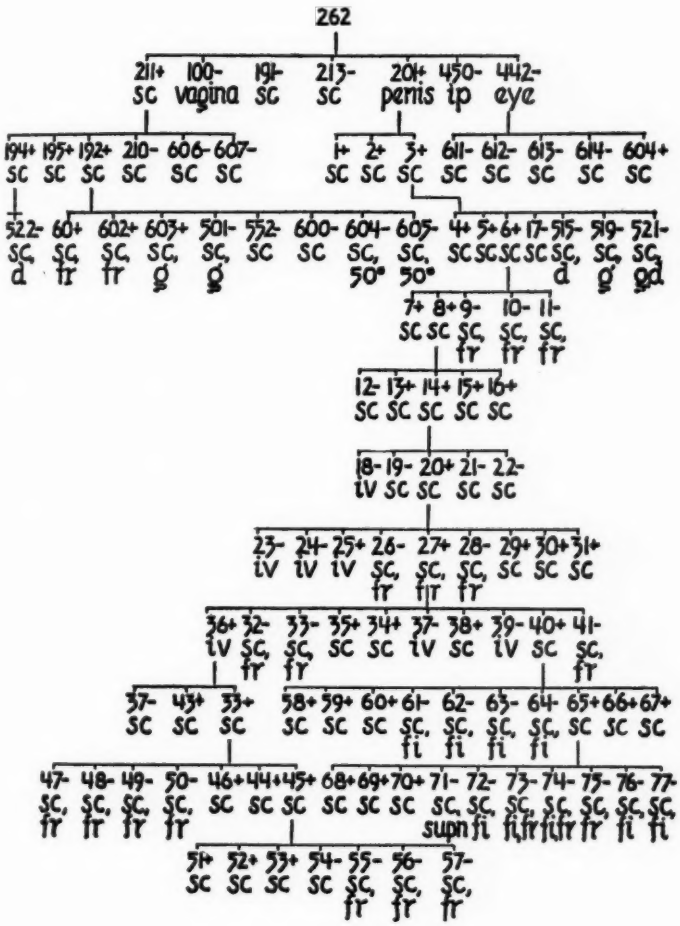

CHART 1. PASSAGES OF A TRANSMISSIBLE STRAIN OF VENEREAL SARCOMA

The following abbreviations have been used:

Route of Injections: sc = subcutaneous; ip = intraperitoneal; and iv = intravenous.

Results: + = inoculation successful; − = inoculation unsuccessful.

Material Injected: fi = filtrate; d = dried; g = glycerinated; fr = frozen and thawed tumor tissue; supn = supernatant liquid obtained by spinning emulsions of cut up tumor tissue.

disappeared. Repeated attempts to reinoculate the animal in which this tumor originated with venereal sarcoma originating in another dog were unsuccessful. Breeding of this dog was attempted 2 years later, when service was satisfactory, but conception did not occur.

The attempted transmission was successful in only 1 dog, which was injected subcutaneously. A tumor appeared at the site of injection and measured about 1 cm. in its greatest diameter, 52 days after injection. The tumor grew slowly, and within 1 month reached the size of about 6 cm. Most of it was removed, and the small part left regressed. Further transfers of this tumor were not attempted.

Transfers were more successful from a venereal tumor on the penis of a pointer (No. 262). This was a soft, gray, cauliflower-like growth approximately 3 cm. in diameter (Fig. 1), with blood oozing from its surface. Blood counts and differential counts were normal. The tumor was successfully transplanted by inoculation into 2 of 7 dogs, and during the course of 3 years 11 successive passages were made. The results of the inoculations are summarized in Chart 1.

Tumor tissue removed aseptically was cut up into small pieces in Locke's solution. Unless otherwise stated, the material injected was an emulsion of tumor cells, the route of inoculation was subcutaneous, and the site of injection the groin. In several instances simultaneous inoculations with different materials were made at different sites.

Subcutaneous Inoculations

An emulsion of tumor tissue was inoculated subcutaneously in 16 experiments and 41 of 57 dogs injected (72 per cent) developed tumors at the site of inoculation. The success of the individual experiments varied from 20 to 100 per cent. In 8 experiments all the dogs inoculated, from 2 to 4 in each, developed tumors. During the first 4 passages 8 of the 19 inoculations (42 per cent) were successful, while in the course of 10 succeeding experiments 33 of 38 (87 per cent) were successful. This increase may be due either to an increased virulence of the strain or to the fact that younger dogs were used. The exact age of these experimental animals was not known, but in the early experiments most of the dogs were comparatively old, in later experiments most of them were young.

The tumor made its appearance in from 18 to 77 days after inocu-

lation. It grew gradually until it approximated from 4 cm. to 10 cm. in the longest diameter. It regressed in most animals from 30 to 250 days after its development was first noted. The tumors were removed in many instances and in none was there any recurrence. The growth was localized to the site of inoculation. Metastasis was observed in only 1 animal. This dog, No. 40, was injected in the left groin with fresh tumor material and in the right groin with frozen and thawed material. A tumor appeared in the left groin 45 days after injection, but none in the right groin. The tumor increased gradually in size for 2 months, and later small tumors appeared in the conjunctiva of the right eye, in the wall of the right thorax, in the subcutaneous tissue in the inguinal region, and at the site of the left popliteal lymph node. Three months later a swelling occurred between the eyes, involving the frontal region. An X-ray picture showed that the skull was not affected, all the tumor being in the soft tissues outside the skull. A small piece of this tumor removed showed the characteristic appearance of venereal sarcoma, as illustrated in Figure 9.

Intravenous Inoculations

In 2 experiments intravenous inoculations were made and 2 of the 7 dogs inoculated developed fatal generalized sarcomatosis. One dog (No. 25) died after an apparent illness of about 3 weeks. Postmortem the dog was emaciated and there were many small tumors in the skin, varying in size from about 1 mm. to 1.5 cm. in diameter. There were numerous tumor masses in and beneath the mucous membrane of the mouth and conjunctiva (Fig. 3). The vagina had prominent metastatic growths measuring about 0.5 cm. in diameter. Subcutaneous tissue and muscles were studded with tumors, as shown in Fig. 2. Tumor masses were found in the intercostal muscles, lungs, diaphragm, heart, pericardium, mesentery, pancreas and kidneys, and several lymph nodes were almost completely replaced by tumor tissue, but the spleen and liver were free.

In another dog (No. 36) tumor nodules appeared 3 months after intravenous inoculation in the mucous membrane of the mouth, in the corium and in the subcutaneous tissue of skin and penis (Figs. 5 and 6). The animal gradually lost flesh and was killed 1 month after the nodules made their first appearance. The postmortem appearances were similar to those of No. 25.

Miscellaneous Inoculations with Fresh Tumor Tissue

The possibility of transmitting the disease through intact mucous membrane was tested by dropping an emulsion of tumor tissue into the conjunctiva of 3 dogs, none of which developed tumors. Tumor tissue was rubbed into the scarified mucous membrane of the penis of a dog (No. 201): a tumor appeared 70 days later and developed into a cauliflower-like growth on the glans penis. This animal is still alive 3 years after inoculation with the tumor, unchanged, as illustrated in Figure 4.

Two dogs were inoculated underneath the mucous membrane of the lip. Small tumors appeared in each. Both tumors regressed and later disappeared.

A fresh emulsion of tumor tissue was injected into the peritoneal cavity of 1 dog and similar material was rubbed into the scarified skin of another dog; neither developed tumors.

Attempts at Transmission by Material Free from Viable Cells

The experiments described below were undertaken for the purpose of determining if the agent transmitting venereal sarcoma is resistant to procedures that destroy mammalian cells but do not affect viruses.

Treatment with Glycerin: Four dogs were injected in 2 experiments with material kept in 50 per cent glycerin, for 10 days in 1 experiment and 4 months in another; none of these animals developed tumors.

Drying: Three dogs were injected with tumor tissue dried *in vacuo* under phosphoric anhydride under conditions that failed to destroy the agent transmitting leucosis [5] of chickens, but all remained free from tumors. Thus attempts to preserve by drying the ability of the tumor tissue to produce venereal sarcoma were unsuccessful.

Filtration: Tumor tissue was cut up in Locke's solution and the centrifugalized supernatant fluid was passed through a coarse siliceous filter. Filtrate thus obtained was injected into the subcutaneous tissue of 2 dogs; the inoculations were unsuccessful. Centrifugalized supernatant unfiltered fluid produced tumors in 2 of 4 dogs injected. The cut up tumor material contained much fat and because of the possibility that fat prevented tumor cells from being thrown down by spinning, this experiment was repeated. Three dogs were injected

with supernatant fluid, but none developed tumors at the site of injection. Two of these dogs (Nos. 69 and 70) were also injected on the opposite side with uncentrifugalized tumor material and a tumor developed in each. Five dogs were injected with the supernatant fluid passed through a very coarse Berkefeld filter and all remained free of tumors. The filter used in this experiment had a bubbling pressure of 23 cm. Hg., and the flow of water during 2 minutes at 40 cm. Hg. was 200 cc.

Freezing and Thawing: Bacteria and viruses are very resistant to low temperatures but mammalian cells, on the contrary, are destroyed when exposed to temperatures below 20° C. Cramer [6] states that sarcoma of mice contains a transmitting agent that resists freezing and thawing, but Furth, Seibold and Rathbone [7] observed that exposure of leukemic lymphocytes to −20° C for 30 minutes abolishes the ability of these cells to transmit leukemia.

In the first 2 experiments 5 dogs were injected with tumor tissue that had been frozen in liquid air. One developed tumors at the site of injection. The test tubes containing the pieces of tumor used in these experiments, however, were not entirely submerged in the liquid air and it is possible that some of the inoculum had escaped freezing. For this reason 4 more experiments were performed in which the material was sealed in a test tube before freezing and was submerged in ether cooled to a temperature of about −70° C by the addition of large amounts of carbon dioxide ice. In these 3 experiments the venereal sarcoma lost entirely its ability to transmit the disease, for none of the 14 dogs injected developed tumors. *B. prodigiosus*, treated in a similar manner in 2 experiments, was unaffected by freezing. The unfrozen emulsion of venereal sarcoma used in these experiments produced tumors in all 13 dogs injected.

Exposure to 50° C: Sticker [8] states that the viability of venereal sarcoma is not destroyed by exposure to a temperature of 50° C for 2 hours. Since mammalian cells are not known to survive such a temperature we have repeated this procedure, injecting 2 dogs with venereal tumor material heated to 50° C for 1½ hours; neither developed tumors.

These experiments present evidence for the assumption that venereal sarcoma cannot be transmitted by cell-free material.

Attempts at Reinoculation

Repeated attempts to reinoculate 2 dogs in which venereal sarcoma underwent spontaneous regression were unsuccessful. One of these dogs was reinjected 3 times. On the other hand, reinoculation was successful in a dog previously injected with tumor material heated to 50° C, and in 3 dogs that had been inoculated with frozen material. These observations support the assumption that the material heated to 50° C, or frozen and thawed, did not contain the agent that transmits venereal sarcoma.

Attempts of Heterotransfer of Venereal Sarcoma to Irradiated Mice

Two attempts were made to transfer venereal sarcoma of the dog to irradiated mice. In 1 experiment 9 mice that had been exposed 4 days previously to 400 r-units of X-rays were inoculated subcutaneously with an emulsion of tumor tissue. In one mouse, killed 9 days after the inoculation, no tumor tissue was recognized grossly, but microscopically (Figs. 13 and 14) tumor cells were seen in the subcutaneous tissue. The tumor cells appeared viable and a few of them were undergoing mitotic division. There was no inflammatory reaction about the area infiltrated by tumor tissue.

In another mouse, killed 11 days after inoculation, there was a tumor of about 2 mm. in size at the site of inoculation. Microscopically this tumor was seen to be surrounded by a connective tissue capsule, through which polymorphonuclear leukocytes invaded the tumor. The central part was necrotic but the cortical part contained apparently healthy tumor tissue (Fig. 15). One of the inoculated mice died 23 days after injection, the rest died or were killed from 6 to 12 days after injection, but none of them showed grossly visible tumors. In another experiment 11 mice irradiated 1 day previously were injected with venereal sarcoma, 8 subcutaneously, 3 intraperitoneally. In 3 mice injected subcutaneously the tumor reached a size of about 2 mm., in 1 about 4 mm. in diameter; the remaining subcutaneous and all the intraperitoneal injections were unsuccessful; 5 of the mice so treated were observed for a period of 2 months and showed on postmortem examination no trace of tumor tissue.

These observations show that cells of venereal sarcoma may survive and multiply for a period of approximately 9 days in the sub-

cutaneous tissue of mice in which resistance has been lowered by exposure to X-rays, but thereafter they undergo regression and disappear entirely.

Discussion

The experiments described indicate that venereal sarcoma can be transmitted only by viable cells. The tumor is characterized by ease of transmission, for it can be successfully passed to dogs of any breed and, according to Sticker,[8] to foxes as well. The natural spread by coitus was described by Smith and Washbourn,[9] and by White.[10] Our experiments show that it is the result of implantation cells, and the tumor has been incorrectly designated "infectious sarcoma of dogs."

Microscopically, venereal sarcoma possesses the characteristics of a malignant tumor; nevertheless sarcomas that are apparently spontaneous disappear or may be completely arrested by removal of most of the tumor. Their disappearance may be explained by assuming that the presumably spontaneous tumors arose by implantation of tumor cells from other diseased dogs through contact. It is noteworthy in this connection that no spontaneous neoplasm of this description was observed in the internal organs.

The origin of the cells forming this tumor is unknown. Evidence for the view that the tumor cells are lymphocytes is wanting. Indeed, they appear to have little affinity for lymphoid organs, their favorite site of growth being the subcutaneous tissue, mucous membranes and corium. The cells in this growth were never seen maturing into typical lymphocytes. For this reason its designation as lymphosarcoma has no basis. Beebe and Ewing [11] consider it to be either alveolar sarcoma or endothelioma. Any discussion of the cellular origin of this tumor is necessarily unsatisfactory. The tumor cells are monotonously alike; they show neither evidence of differentiation nor of organization. There is no evidence that they are reticular cells. Reticulum fibers are few if any among the tumor cells. The tumor cells, when detached, are obviously cytologically different from histiocytes. Anastomosis or formation of syncytium was not seen. Phagocytic properties were not observed, although this was not tested experimentally. Lack of formation of vascular channels or cavities by tumor cells and failure to demonstrate origin in endothelium are sufficient reason for not designating this tumor an

endothelioma, although the endothelial origin of the tumor cells is the most likely supposition, according to Ewing.[12]

This tumor may apparently arise in locations other than the genital organs. Feldman has described a tumor originating in the eye, indistinguishable microscopically from venereal sarcoma. One of the spontaneous instances of this disease observed at the veterinary school originated in the skin or subcutaneous tissue over the anterior tibial region of a male Boston terrier.

The tumor-like growth of rabbits caused by filterable viruses (myxoma of Sanarelli, *cf.* Ref.[13]) and the similar tumor described by Shope [14] differ from venereal sarcoma in two respects: (1) the agents of the former tumors readily pass bacteria-tight filters and can be preserved in glycerin; and (2) they originate from the cells of the host that are stimulated to rapid reproduction by a filterable virus. Venereal sarcoma caused by transmission is the result of multiplication of the transplanted tumor cells (Beebe and Ewing [11]) and all attempts have failed to transmit the tumor by material not containing viable cells.

Summary and Conclusions

Two venereal sarcomas have been successfully transmitted to healthy dogs, and one of them was transplanted in 11 successive generations.

Inoculations were successful in 72 per cent of the dogs injected subcutaneously with emulsion of tumor cells. Tumors appeared at the site of inoculation within an average of 38 days and with one exception began to regress after reaching a size of about 10 cm. in the longest diameter. In 1 dog the tumor spread by metastasis throughout the body.

Intravenous inoculation produced generalized sarcomatosis in 2 of 7 inoculated animals. Transmission was also successful by rubbing tumor material into the scarified surface of the glans penis. Attempts to transmit the disease through intact mucous membrane (the conjunctiva) were unsuccessful.

The ability of the tumor material to transmit the disease was destroyed by the addition of 50 per cent glycerin, by desiccation, by freezing and thawing, and by heating to 50° C for 1½ hours. Tumor material passed through siliceous filters likewise failed to produce tumors.

These experiments indicate that venereal sarcoma, often designated "infectious sarcoma of dogs," is a neoplastic process and, like other mammalian tumors, can be transmitted only by viable tumor cells.

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DESCRIPTION OF PLATES

PLATE 81

FIG. 1. Spontaneous venereal sarcoma on the penis of a pointer (No. 262) in the form of a soft cauliflower-like growth of approximately 3 cm. in diameter.

FIG. 2. Tumor nodules in the subcutaneous tissue and muscles of a dog (No. 25) caused by intravenous injection.

FIG. 3. Tumor nodules beneath the mucous membrane of the mouth and conjunctiva caused by intravenous inoculation (No. 25).

FIG. 4. Cauliflower-like growth of 3 years duration on the penis of a poodle, produced by rubbing tumor tissue into the scarified mucous membrane of the penis (dog No. 201).

FIGS. 5 and 6. Generalized tumor formation in the skin and subcutaneous tissue produced by intravenous injection (dog No. 36).

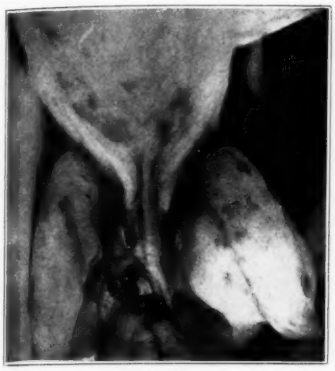

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PLATE 82

FIG. 7. Microscopic appearance of venereal sarcoma of dogs with scant amount of connective tissue. Hematoxylin and eosin. × 100.

FIG. 8. Microscopic appearance of venereal sarcoma with abundant connective tissue separating the tumor cells into small nests. Hematoxylin and eosin. × 100.

FIG. 9. Same as Fig. 7, higher magnification. × 400.

FIG. 10. Same as Figs. 7 and 9, higher magnification. × 900.

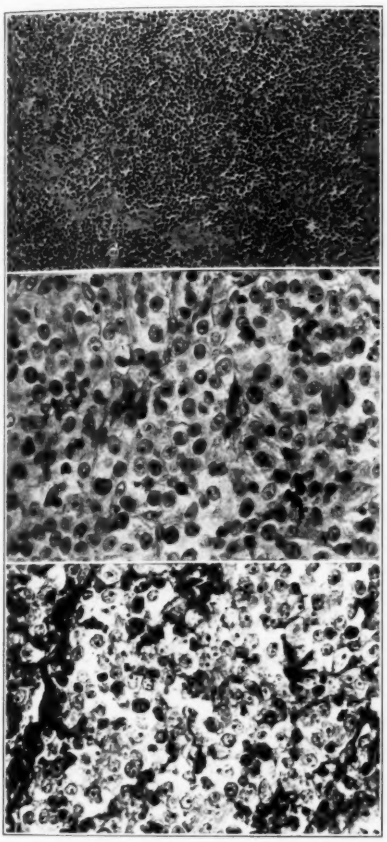
FIG. 11. Section of venereal sarcoma stained with Heidenhain's modification of Mallory's anilin blue stain, showing that fiber formation is abundant about the blood vessels, but scant if any among the tumor cells. × 400.

FIG. 12. Reticulum fibers in venereal sarcoma reproduced according to Foot's silver impregnation method. × 200.

FIG. 13. Microscopic appearance of a small tumor nodule of venereal sarcoma of dogs 9 days after subcutaneous injection in an irradiated mouse. × 200.

FIG. 14. Same as Fig. 13, higher magnification. × 600.

FIG. 15. Venereal sarcoma of dogs 11 days after subcutaneous inoculation in an irradiated mouse. The tumor is surrounded by a thick fibrous capsule. Its central part is necrotic and leukocytes invade the peripheral part, which is composed of apparently viable tumor cells. × 200.



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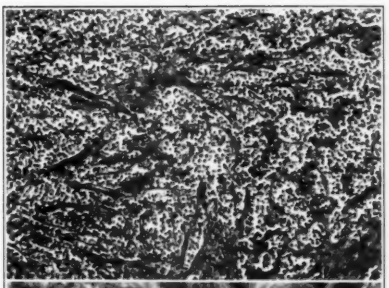

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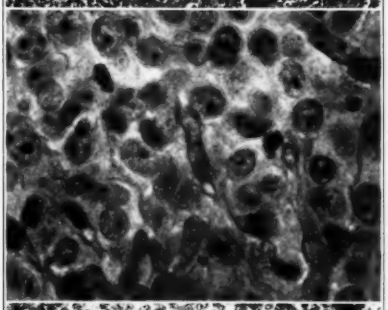

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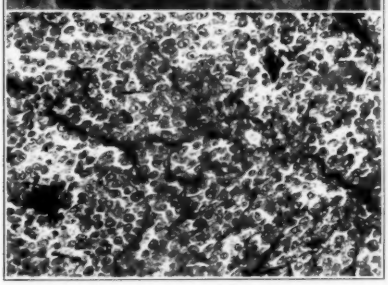

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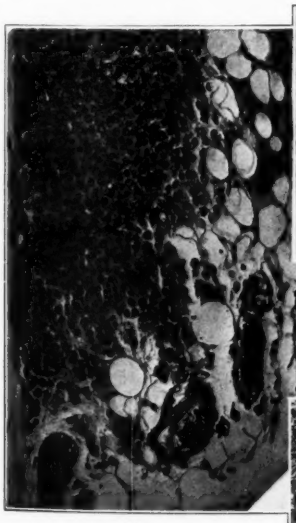

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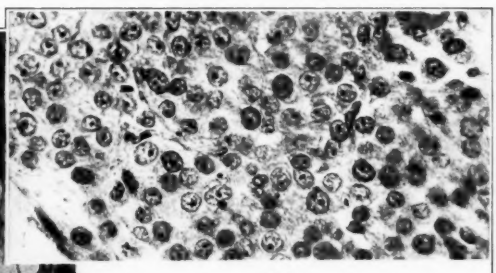

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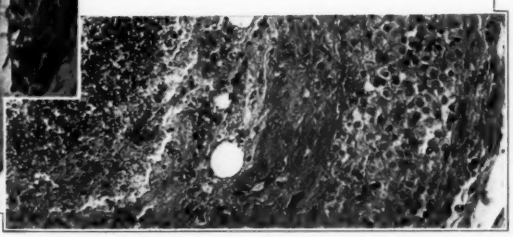

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Stubbs and Furth

Venereal Sarcoma of the Dog

THE RENAL LESIONS OF RHEUMATIC FEVER *

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While for many years it has been recognized that rheumatic fever exerts a profound influence upon the various structures of the heart it is only recently that the possibility of the far reaching effects of this disease in other organs, and particularly in vascular tissues, has been recognized. In what manner and to what extent the kidney may be involved in rheumatic infection it has been the purpose of this study to investigate.

Although the fully developed Aschoff nodule, as seen characteristically in the myocardium, is probably the only truly specific histological expression of rheumatic fever, its presence must be regarded as representing merely one particular phase in the course of a morbid process, and as such must probably be looked upon only as an extreme manifestation of a general mode of reaction. It must be emphasized further that the rheumatic nodule cannot be regarded as a fixed or static structure, that it is undergoing continual changes, and that it probably passes through a fairly definite life cycle. That closely similar, though less specific, local inflammatory reactions may occur in relation to the smaller vessels, particularly in the kidney, we shall endeavor to point out.

Considerable attention has been devoted to this problem by various writers, the observations of Fürbringer, Baehr, Sacks, Löhlein, Fahr, Pappenheimer and VonGlahn being particularly worthy of note. Klotz, however, was the first to draw attention to the constancy with which widespread lesions occurred in the arterioles of the viscera in rheumatic fever. In the kidney he observed a non-suppurative, perivascular infiltration in relation to the smaller vessels, comparable in character to that found in the myocardium. This began in the vicinity of the intralobular arteries and spread along the course of the vessels into the cortex. Healing was observed to take place by a radiating type of fibrosis with later shrinkage and the production of a granular contracted kidney. He was able to

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produce similar perivascular lesions in the hearts and kidneys of rabbits by the intravenous injection of various types of streptococci.

In the present study 16 proved cases of rheumatic fever were selected, 10 of these, ranging from 18 to 52 years of age, having come to autopsy in the Toronto General Hospital, and 6 at the Hospital for Sick Children (3 of these were 3 years of age, the others 8, 10 and 13 years respectively). Definite Aschoff nodules were demonstrated in the myocardium in 13 of the 16 cases.

In the majority of instances the lesions to be described in the kidney were fairly distinctive and were present in 14 cases. These changes occurred characteristically in relation to the smaller vascular structures, particularly to the intralobular and arcuate arteries and the arterioles of the cortex. They were never observed in relation to the larger branches of the renal vessels. In general, the pathological changes were of three types. Evidence of acute or subacute inflammation was present in 8 of the 16 cases, a chronic or healed lesion was found in 4, while a recurring type of inflammatory reaction was met with twice. The various pictures may be described separately.

Acute Non-Suppurative Periarteritis

The lesion consists essentially of a non-suppurative type of inflammation in the perivascular spaces involving the adventitia and with frequent pathological changes in the medial and intimal coats. The inflammatory exudate is made up chiefly of lymphocytes and plasma cells, together with a few indefinitely outlined cells, the nuclei of which are pale staining, vesicular and sometimes distorted in appearance. Occasional polymorphonuclear leukocytes with fragmented nuclei are also found. The stroma surrounding many of the involved vessels presents a peculiar appearance, being composed of a fine fibrillar type of connective tissue with few nuclei. In many cases the fibrils are loosely arranged and widely separated from one another, giving the appearance of a perivascular edema.

The inflammatory reaction frequently surrounds and involves only a portion of the vessel and has a somewhat nodular arrangement suggesting the focal type of reaction so often seen about the small vessels in the myocardium. In some specimens, however, the zone of inflammation surrounds the entire vessel. The inflammatory

cells lie for the most part in the periadventitial and adventitial coats, the exudate spreading apart the connective tissue fibers and sometimes replacing them. Usually the structures of the adventitia appear indefinite and have the fibrillar and edematous appearance noted above. The picture has often the appearance of a granulomatous type of reaction and has been referred to as such by Fahr. Frequently the inflammatory cells are confined to the adventitia and periadventitial structures; in some instances, however, the media is also involved. Here the same type of cells is seen lying between the muscle fibers, the normal structure being replaced by a loose fibrillar type of tissue similar to that seen in the adventitia. Many of the nuclei of the muscle cells have disappeared in that portion of the vessel most involved in the inflammatory reaction; other nuclei appear swollen or vesicular in character with loss of chromatin substance. Frequently nuclear fragments lying free in the vessel wall among the inflammatory cells are seen. The muscle cells may appear in various stages of disintegration and dissolution with considerable thinning out of the medial coat in the most involved portion of the wall. At times these changes involve the entire circumference of the vessel and occasionally complete or almost complete disintegration and necrosis of the wall has been observed.

The intimal coat of the involved vessel shows no constant or characteristic change. In the larger vessels small nodular areas of endarteritic thickening are not uncommonly met with, but are never extreme. In those vessels, however, that show marked involvement of the medial coat advancing to necrosis and disintegration of the muscle cells, an intimal change is usually noted. This consists in a marked swelling of the lining endothelial cells, which may show large rounded vesicular nuclei and pale staining cytoplasm. Sometimes in the smaller vessels this swelling is so marked that the lumen is markedly encroached upon.

Sections were stained for fibrin by the Gram-Weigert method and for elastic tissue by both Weigert's and Verhoeff's stain. A careful search was made for the lesions described by Pappenheimer and VonGlahn, namely, an exudation of fibrin beneath the endothelium and sometimes within the muscle wall, with later canalization and organization of the exudate. This lesion, which they describe as being specific, was never encountered in any of our sections. The stretching and apparent fragmentation of the internal elastic lamina

also mentioned by these observers was noted in isolated instances, but was always accompanied by an endarteritis.

The intimal lesions later described by these authors, consisting of rows or palisades of cells separated by bands of fibrin-like material and associated by these observers with rheumatic lesions in the auricular wall, were also never found. The perivascular lesions which they describe, however, have in general a resemblance to those found in the present investigation, although the latter have a more localized or nodular arrangement than the former.

Healed Perivascular Lesions

The exudative lesions described above represent the active phase of the inflammatory process and may justly be referred to as a nodular type of non-suppurative periarteritis. With this is associated at times a mesarteritis with partial destruction and disorganization of the muscular coats of the vessel and a variable degree of reaction in the intima. Evidence that this lesion, like all perivascular rheumatic inflammatory changes, heals with the formation of scar tissue is not lacking. Various stages in this process may be observed in the sections examined. As the acute phase of the inflammatory reaction subsides the edema surrounding the vessels disappears along with many of the inflammatory cells, fibroblasts make their appearance and the loose fibrillar stroma is gradually converted into dense scar tissue. In some of the cases studied this perivascular scarring occurs in a radiating manner in wide zones about the vessels. Frequently, irregular, wedge-shaped processes of scar tissue extend out from the vessel into the adjacent parenchyma, which to some extent is encroached upon and replaced. Not infrequently isolated glomeruli or tubular structures are seen completely surrounded by wide bands of fibrous tissue extending out from the neighboring vessels. Later, the areas of scarring become less cellular and large amounts of collagen material are laid down. With contraction of this scar tissue deformity of renal structures occurs, with finally the production of a shrunken and granular kidney. In 4 cases the organs in the gross showed a slightly thickened capsule and a markedly granular surface; in 1 of these the process was unusually well defined. In 4 others the surfaces only presented a slightly granular appearance. In none was the size of the organ considerably reduced.

In the reparative processes the adventitial coats of the affected vessels become involved in scar formation. The media, however, seems to undergo a peculiar hyaline change, and in the smaller vessels is frequently seen to be almost devoid of nuclei and to be represented by a homogeneously acellular hyaline mass, poorly differentiated from the adventitia.

Recurrent Perivascular Inflammation

In 2 of the cases studied definite evidence of a recurring process is present. Recent and acute cellular infiltration, often quite localized and focal in character, is met with in areas of old perivascular fibrosis in which considerable collagen material has been deposited. Evidence of an accompanying edema is not marked but frequently areas in which necrosis of collagen fibrils has occurred in relation to the inflammatory process are noted.

Several times evidence of what appears to be a purely degenerative change, not directly associated with any of the above lesions, is present in many of the small arteries and arterioles. This is particularly well seen in 2 cases in which the walls of many of the finer vascular structures are considerably thickened and completely or almost completely replaced by pink-staining, granular, acellular material. The muscle fibers of the media have lost their identity and the nuclei have disappeared. The picture suggests necrosis of the vessel wall, associated with the inflammatory lesions described above. In some instances a hyaline-like change within the wall is noted. Moreover, considerable thickening of the afferent glomerular arteriole also is sometimes present. Frequently, but not invariably, associated with this is a well marked change in the corresponding glomerulus, the latter showing an atrophy, collapse and hyalinization of the capillary loops and occasionally of the entire glomerular tuft. This picture has been noted by many observers, occurring both in rheumatic and in non-rheumatic conditions. Its significance, however, is little understood, some claiming it to be purely secondary and dependent upon glomerular changes, others being of the opinion that the lesion is of primary importance in bringing about atrophy of the capillary loops through nutritional disturbances. Fahr has noted an inflammatory infiltration about this region of the afferent arteriole and describes it as being of a

granulomatous character, leading to closure of the lumen and necrosis with later hyalinization of the wall. This, although aptly describing the changes observed in many of the smaller vessels and arterioles in the cases under discussion, was not observed at the point of entrance to the afferent vessel.

Glomerular Changes

As pointed out by many observers, the occurrence of acute glomerulonephritis in association with rheumatic fever is rare. In the present series this was never observed. Glomerular damage is present only occasionally. Only once was evidence found to suggest an inflammatory lesion of the glomeruli. This appears in a child (C–152–32) of 8 years, dying in about the third week of illness, with evidence of a subacute inflammation in occasional glomeruli. In none of the others is any endothelial or epithelial proliferation of the glomerular structures noted. Inflammatory infiltration and capsule adhesions are entirely absent. In only a few, where a glomerulus lies in close proximity to a focus of inflammation about a vessel, is there any fibrous thickening of Bowman's capsule or any swelling of the lining endothelial cells. The glomerular damage when present consists of hyalinized structures, occurring singly or at times in groups. Those glomeruli lying directly beneath the capsule are most frequently affected and small roughly wedge-shaped areas of scarring containing one or more hyalinized glomeruli are occasionally seen, representing areas of early arteriosclerotic atrophy. The involved glomeruli throughout the section are seen to be undergoing a bland type of hyalinization without, as mentioned above, showing evidence of a previous or co-existing inflammatory change. This observation lends strong support to the view that the glomerular damage present in these cases is not primarily of inflammatory origin, but rather secondary to and dependent upon nutritional disturbance brought about by vascular change.

Tubular Changes

Relatively little alteration is noted in the tubular structures apart from occasional atrophy and disappearance of those associated with obliterated glomeruli. At times scar tissue extending outward from a vessel is seen to surround and distort adjacent

tubular structures. A moderate nephrosis is present in 1 case. Sometimes granular débris and occasional hyaline casts are noted in the tubules. Well marked passive congestion is often present.

Additional Cases Reviewed

In addition to the above study all cases presenting evidence of rheumatic heart disease coming to autopsy during the last seven years were reviewed. Out of 2400 autopsies, 128 (5.3 per cent) were found showing either healed or active cardiac lesions. Of this group interstitial nephritis had been diagnosed in the gross in 38 (30 per cent). Half of these had occurred in individuals under 40 years of age and one-third of them in those under 30 years of age. These figures serve to illustrate two points: first, that a very definite association exists between rheumatic fever and renal disease, and second, that a high percentage of these individuals show evidence of chronic kidney damage at a very early period in life. Arteriosclerotic atrophy of the kidney, on the other hand, which was met with in 19 of the 128 cases (15 per cent) occurred in the great majority beyond the age of 50. This is in keeping with the observation that only the smaller arteries and arterioles are involved in the pathological process under discussion.

Discussion

From a review of the literature it is apparent that involvement of the vascular system is a common accompaniment, if not indeed a constant manifestation, of rheumatic infection. While it has been shown that a very close relation exists between lesions appearing in the heart and arterial system, the degree of pathological alteration observed in any particular organ is subject to wide variation. It is clear, however, that the lesions induced by a rheumatic infection have certain well defined and characteristic manifestations in whatever tissue or organ they may be found, and that a common mode of reaction is always present. The most common and characteristic expression of rheumatic infection is in a non-suppurative perivascular reaction affecting chiefly the smaller vessels, associated with edema and round cell infiltration, and leading to the formation of new connective tissue. We are becoming more and more convinced that the pathological changes taking place in this condition are largely dependent upon a primary vascular lesion.

It would appear that this reaction and its influence upon the kidney structure constitutes a definite type of interstitial nephritis. While the balance of evidence would suggest that the glomerular change is a result of a nutritional disturbance and not primarily of inflammatory origin, it is easily conceivable that the same injurious agent that is responsible for the vascular lesion might occasionally lead to direct glomerular damage. As Klotz long ago pointed out, vascular changes in the kidney do not occur apart from inflammation, and it is to a common type of injury that both vascular structures and parenchymal tissue react.

While we believe that the changes noted are of frequent occurrence and give rise to a definite type of interstitial nephritis, the renal damage is only occasionally of sufficient severity to attract the attention of the clinician and lead to a diagnosis of kidney disease during life. In the acute stages of rheumatic fever small traces of albumin in the urine, which are frequently encountered, are usually ascribed to other causes. On the other hand a failing heart, with an associated congestion of the kidneys, very materially obscures the picture of an underlying nephritis toward the terminal stages of the disease. It must be observed, however, that in recurring infections the repeated injury, which plays such an important rôle in the pathological changes within the heart, may in a similar manner be suffered by the kidney, leading to a gradually diminishing reserve in the functioning power of that organ.

We cannot, however, assert that the changes which we have described in association with rheumatic fever may not be clearly reproduced by other forms of infection, particularly those of streptococcic origin. None of the lesions described as specific manifestations in the kidney by certain authors, particularly Pappenheimer and VonGlahn, has in our experience been observed. It would seem, however, that a mode of reaction in general similar in character and comparable to that found in the myocardium and wall of the aorta may take place in many of the peripheral vessels. Until more light has been thrown upon the etiology of rheumatic fever the part played by this disease in nephritis is open to debate. That such a relation exists is, we believe, beyond question. It must be emphasized that the changes observed have an irregular distribution, and that, as a rule, only relatively few vessels are involved in the pathological processes described. Thus, in general, insufficient

damage is brought about to impair renal function seriously, and it is only exceptional that evidence of kidney disease is discovered during life. It must be stated definitely, however, that in a large percentage of cases of rheumatic fever the kidneys, as well probably as many other organs, do not entirely escape, and will exhibit evidence of acute, recurring or chronic vascular damage, reflecting itself in a varying degree of pathological alteration of renal structure.

Summary and Conclusions

1. A study of the kidney lesions in 16 cases of rheumatic fever was undertaken.

2. A perivascular inflammatory reaction of the acute non-suppurative type, affecting the smaller arteries and arterioles was present in 8 cases. Evidence of perivascular scarring was noted in 4 cases, while a recurrent type of inflammation was met with in 2.

3. The inflammatory reaction is usually seen in the adventitia and periadventitial tissues, with occasional infiltration and destructive change in the medial coat. Intimal changes, consisting of an endothelial swelling and proliferation, are inconstant.

4. Glomerular damage, which was only well marked in 1 case, is to be regarded as dependent chiefly upon nutritional disturbances brought about by vascular changes. Little evidence of active or healed inflammatory processes was met with in the glomeruli.

5. No evidence of the specific vascular lesions described by Pappenheimer and VonGlahn was met with in the cases studied.

6. The lesions described, which in general bear a close resemblance to perivascular foci of inflammation found in the myocardium, may be looked upon as constituting a definite type of interstitial nephritis. It is seldom, however, that sufficient alteration in structure to justify a diagnosis of renal disease during life occurs.

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PRIMARY AMYLOID DISEASE OF THE HEART *

REPORT OF A CASE

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Although primary amyloid disease of the heart is very uncommon, the cases that have been reported in the literature [1-4] indicate that the hyaline substance may be deposited in the epicardium, myocardium, endocardium, valves, or in the walls of adjacent blood vessels. Pronounced involvement of the endocardium, the superior and inferior venae cavae and the pulmonary artery was found in a case seen recently. Since the literature on this subject does not include illustrations of lesions having the distribution indicated it is the purpose of the present communication to record pictorially amyloid infiltration of the vascular lining of the heart and blood vessels. Also, Mayer's method of staining amyloid in paraffin sections [5] is described because it has been found worthy of more general application.

REPORT OF CASE

Clinical History: A 75 year old white male was admitted to St. Vincent's Hospital, Los Angeles, on July 13, 1932, with the chief complaint of hematuria. He had suffered from nocturia three times nightly for some time and there had been difficulty in starting the stream. Urination was not painful, although it was slow and the stream was intermittent. On June 30, 1932, dark red clots of blood appeared in the urine, which cleared up after four to five urinations and remained clear until the night before admission when bloody urine was again noticed. There was never any pain although there had been soreness above the symphysis. The patient had had typhoid at 18 years of age, malaria at 20, "kidney colic" at 40 and inflammation of the gall-bladder 1 year ago. There were no cardiac complaints.

Physical examination revealed a well preserved, elderly male who weighed 210 pounds. The blood pressure was 130/72 and the pulse 80. Examination of the head, neck, chest and abdomen gave negative results. Upon rectal examination the right lobe of the prostate was 4 plus enlarged, firm and nodular, while the left lobe was smaller in size and smooth over the surface. The bladder contained 75 cc. of residual urine. Cystoscopic examination of the bladder revealed old and recent blood clots that obscured the base, but the lateral walls and dome were normal.

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The urine was free of sugar but showed a trace of albumin with a few erythrocytes and pus cells. The hemoglobin was 70 per cent (Sahli) and the white blood cells numbered 10,800 with 73 per cent polymorphonuclear leukocytes. The blood urea was 34 mg. Roentgenological examination of the chest showed the heart shadow moderately enlarged but the lung fields were normal. Films of the kidneys, ureters, bladder and pelvic bones were negative for calcareous deposits and metastases.

The diagnosis of carcinoma of the prostate gland was at first considered, but following cystoscopy it was thought that the bleeding was due to an enlarged, benign, intravesical prostate. Suprapubic drainage of the bladder was advised and was carried out. A tumor 3 cm. in diameter was found situated in the base of the bladder anterior to the right ureteral orifice, with two secondary nodules about 1 cm. in diameter to the right of the internal urethral orifice. A segmental resection of the bladder, including the tumor, was made and the bladder reconstructed. The lesion was reported as carcinoma of the bladder.

The patient reacted satisfactorily to the operation and the postoperative course was essentially uneventful. The blood pressure remained about 140/60. The pulse remained full, although there was some irregularity with occasional extra systoles. He was discharged from the hospital July 19, 1932, wearing a urethral catheter, and there was still some drainage from the suprapubic wound. He was readmitted to the hospital Nov. 7, 1932, nearly 5 months after operation, because of suprapubic extravasation of urine and inability to get along without the urethral catheter. Examination of the heart at this time revealed a blowing systolic murmur over the aortic area, which was also heard over the apex and was transmitted to the axilla. Suprapubic drainage was again instituted and extensive recurring carcinoma was found in the bladder. He returned home Nov. 18, 1932, where he remained until his death, May 8, 1933. During this interval hard tumor masses appeared in the abdomen, which attained a very large size. There were symptoms and findings suggestive of bilateral ureteral obstruction with marked urinary sepsis.

An autopsy was performed 12 hours after death. Since the lesions that constitute the subject of this report were found solely in the heart and great vessels, only these structures are described in detail.

Gross Pathology of Heart

The heart weighed 500 gm. and was symmetrically enlarged. The serosal surfaces were smooth and glistening and a considerable amount of subepicardial adipose tissue largely obscured the musculature. This extended well upward over the pulmonary artery and systemic aorta. Upon exposing the endocardium of the right auricle the surface was covered by numerous closely placed translucent nodules that were scarcely more than pin-point in size. Upon touching them lightly with the finger tips they were easily palpated and imparted the sensation of a finely sanded surface. The identity of this process was not appreciated until after microscopic sections

were studied, when it became apparent that the glassy material was amyloid. Its distribution could be more accurately estimated after staining the heart with iodine (Fig. 1). Nodules extended over the surfaces of the superior and inferior venae cavae for a distance, but there seemed to be a rather sharp line of demarcation in the superior vena cava, beyond which the vessel was normal. Amyloid was also quite conspicuous over the valve of the coronary sinus, as well as in a Chiari network,[6] which coursed along the superior surface of the auricle from the left margin of the superior vena cava. The foramen ovale was widely patent and the thin membrane that guarded the opening was covered by conglomerate foci. The leaflets of the tricuspid valve and even the chordae tendineae were surprisingly free of amyloid. Nodules were quite numerous in the endocardium of the right ventricle, however, (Fig. 2), being most conspicuous just below the pulmonary valve. The leaflets of the valve were quite free of disease, except close to the line of attachment where occasional stained areas were present. From the valve almost to the bifurcation the intima of the pulmonary artery was peppered with amyloid, but distally a normal structure was assumed. The endocardium of the left auricle (Fig. 3) showed smaller and probably fewer foci of hyaline material than the right auricle, but maximum involvement in both was present in the membranous interauricular septum. Practically no amyloid was found in the mitral valve leaflets, chordae tendineae, or on the surface of the papillary muscles, although several glistening, translucent vegetations 2 to 3 mm. in diameter were present along the free edge at the left angle of the valve and along the midportion of the anterior leaflet. Nodules of amyloid were less numerous in the endocardium of the left ventricle. Upon exposing the aortic valve there were a considerable number of rounded, calcified masses 2 to 4 mm. in diameter present over both surfaces of all the leaflets, and calcified adhesions were found between the free edges of the leaflets at the right anterior commissure for 0.8 cm. from the aorta. There was no calcification in the aortic wall of the sinuses and the intima of the aorta showed only occasional streaks and elevated plaques of yellowish, opaque, atheromatous material. No amyloid was seen in either the valve or the artery. The coronary orifices were unobstructed and when the vessels were followed by serial cross-sections the walls showed only moderate atheromatous changes. No amyloid was found in the intima or media of the major

arteries, although the walls of the coronary veins were heavily infiltrated. The myocardium throughout was somewhat pale but moderately firm and uniform in consistence and texture. There was but the slightest suggestion of a glassy mottling in the unstained tissue, but a section through the midportion of the left lateral wall of the left ventricle, when stained with iodine, showed a considerable amount of amyloid in the myocardium (Fig. 4). This was also true of the right ventricle and the walls of both auricles.

Microscopic Examination

A small piece of endocardium from the left auricle was stained in iodine and examined with the low power objective, using reflected light (Fig. 5). The surface showed a mosaic pattern, being divided into oblong, rhomboid and polygonal areas less than 1 mm. in diameter by sharp sulci, which intersected at all angles. Occasionally the grooves were parallel and formed longitudinal folds. The surface amyloid was largely limited to the summits of such areas and although it extended down over the margins for a distance in the zones of heavier deposit, it was uncommonly found in the troughs. In its finest form it was deposited as rounded, sharply circumscribed nodules 50 to 100 microns in diameter. Where these were closely placed they were likely to coalesce and the fused nodules formed bizarre patterns of various sizes and shapes which might be triangular, rod-like or quite rounded. Where coalescence had resulted in larger accumulations the amyloid was distributed in some variation of star-shape. Some were quite perfect six-pointed stars that presented a stippled appearance; others showed a central nucleus about which there were bar-like striae radially placed. The summit of a longitudinal fold, which was covered by many closely packed star-shaped masses of amyloid, was not unlike the appearance of a chain of mountains on a relief map. The various figures and patterns that could be found were limited only by the imagination of the observer. Myocardium from the left auricle, which was cut parallel to the surface, was similarly studied (Fig. 6). There were bundles of fine, white, opaque fibrils that ran in many directions and formed a tightly woven meshwork, the interstices of which contained vacuolated tissue. This meshwork of fibrils showed a tigroid mottling of alternating dark brown and pale yellow zones, which were short, broad and feather-edged. Closer inspection showed the

deeply staining bands crossed by fine white parallel fibers, which varied in caliber from one place to another and which anastomosed and branched. The paler areas were striated by fine brown streaks that often connected one dark zone with another. It was obvious that the pale fibrils were muscle cells and the dark material was amyloid. In the dark zones thin muscle fibers were seen against a dark background of stained amyloid, and in the light areas narrow streaks of amyloid were seen against a pale background of muscle tissue. The amount of amyloid varied from place to place and alternating zones of about equal size contained large and small quantities. A fine white stippling over a dark brown field was noted in the rare areas in which a muscle bundle was seen in cross-section. In the adipose tissue the individual fat globules were brought into sharp relief by amyloid that was deposited between cells.

Tissue for paraffin sections was taken from many areas of the auricles, ventricles, valves and large blood vessels, some of the typical lesions of which will be described.

Pulmonary Artery: Rounded, ovoid and lenticular areas of hyaline material are found in the intima, media and adventitia (Fig. 7). They are quite dense, homogeneous, acellular and sharply demarcated as a rule, being more numerous superficially, with many bulging toward the lumen from just beneath the internal elastic membrane. Although elastic fibers can be traced coursing through an area they usually end rather abruptly at the margin. The internal elastic membrane sometimes splits to surround a nodule.

Endocardium: In the endocardiun of the auricles amyloid is deposited in much more irregular plaques which vary greatly in size and have a tendency to spread along the surface and coalesce (Figs. 8, 9 and 10). Many plaques which extend to the surface are covered only by endothelium. Some present a palisade effect and are the full thickness of the endocardium, while others are only in the deeper layers.

Myocardium: The amyloid has a very patchy distribution. It can easily be identified appearing in the fibrous stroma and frequently is closely applied to the individual muscle fiber, forming a sheath or tube of narrow or broad dimension which completely encircles the cell. In places it is seen only in the walls of blood vessels and the stroma itself is free. The muscle fibers in many instances appear of normal size and their finer structures are well

preserved. Atrophy is noted, however, in foci of more dense accumulation with some fibers entirely missing, leaving unstained vacuoles in a mass of amyloid. Involvement of the musculature near the epicardium seems no different from that of the central part or near the endocardium, although it is most extensive in the wall of the right auricle with the left auricle and the right and left ventricles next in order.

Pericardium: Amyloid is found in two locations, chiefly close to the myocardium, where it has been deposited between the fat cells to a moderate degree. It is also seen in the walls of smaller vessels, especially veins. Plaques of amyloid beneath the serous surface are not found.

The other postmortem findings were largely those of extensive neoplastic involvement of the prostate, bladder, suprapubic sinus tract, retroperitoneal tissues, lymph nodes, kidneys, pancreas and mesentery. There was obstruction of both ureters with marked hydronephrosis on the left and pyonephrosis on the right. No metastases were demonstrated in the liver and lung, or in the vertebrae and pelvic bones exposed. The tumor microscopically is an adenocarcinoma, being primary in the prostate. There is no microscopic evidence of amyloid in the lung, spleen, liver, pancreas, colon, adrenal, kidney, bladder, prostate, lymph node or tumor tissue. An examination of the nervous system was not made.

Pathological Diagnoses: Extensive carcinoma of prostate with extension to the bladder, suprapubic sinus tract, retroperitoneal, periaortic and mesenteric lymph nodes, kidneys and pancreas; amyloid disease of the heart, pulmonary artery and venae cavae; endocarditis, old with aortic stenosis; endocarditis, acute (mitral); hydronephrosis with pyonephrosis (right); acute ulcerations of stomach; old cholecystitis with mucocele; multiple infarctions of spleen.

Methods

The stains employed to identify amyloid were iodine and gentian violet. For gross staining the whole heart was placed in a 5 per cent alcoholic solution of iodine and when well stained was washed in water to which a few drops of sulphuric acid were added. About 3 days were required for complete destaining and photographs were

taken when the desired degree of contrast appeared, which was after 24 hours in this instance. In photographing the specimen a strong yellow filter such as the Wratten "G" helped the contrast, amyloid appearing black in the picture.

Paraffin sections were stained with gentian violet after Mayer's method, as described by Mallory and Parker.[5] Formalin-fixed tissues were embedded in paraffin and cut into suitable ribbons. One, two or three slices were cut from the ribbon with a scalpel and allowed to spread by transferring with a camel's hair brush to a small dish of distilled water which had been heated to 108° F. When completely spread they were pulled upon a glass slide and transferred to a warmed 0.5 per cent aqueous solution of gentian violet and allowed to stain 2 to 5 minutes. Staining was controlled with the microscope and as soon as the amyloid had a good pink color, with the remaining tissue purple, the sections were quickly washed in warmed distilled water and allowed to differentiate in ¼ to ½ per cent acetic acid. Differentiation was also controlled microscopically and as soon as completed, usually in 30 to 60 seconds, the sections were quickly washed in fresh warm distilled water and mounted on clean glass slides without using glycerin-albumin. Drying over night at room temperature was usually sufficient, although it was sometimes necessary to warm in the 37° C incubator. After thorough drying the sections were dipped in fresh xylol 1 to 2 minutes to deparaffinate and clear and were mounted in gum damar.

The procedures can be very conveniently carried out by employing small oblong staining dishes of about 100 cc. capacity in which the solutions are kept at the proper temperature on a hot plate. In the absence of a constant temperature hot plate the jars were placed in a 12 inch pan filled with water which was kept at 105° F by a small alcohol lamp. It was easy to transfer the paraffinated sections from solution to solution by pulling them onto a glass slide with a dissecting needle or camel's hair brush.

When the sections were placed directly in the stain, without first allowing them to spread, the stain was likely to be uneven. Overstaining destroyed the contrast between amyloid and fibrous tissue and overtreatment with acid resulted in very pale, poorly contrasting sections. Moisture interfered with the preservation of the stain and could easily be detected before deparaffinating by examining the section with the low power objective, using diminished illumi-

nation. A strong green filter such as a Wratten "B" was most useful in photographing the gentian violet stain, amyloid appearing black in the picture.

Discussion

Cases of amyloid disease of the heart fall into one of three general classes, depending upon the deposition of the hyaline substance in other tissues and organs of the body. In the largest group of cases of generalized amyloidosis, diffuse infiltrations affect various parenchymatous organs, such as the liver, spleen, kidney, adrenal, pancreas and, not infrequently, the heart. In generalized amyloidosis of muscular systems,[7,8] there is an atypical distribution of amyloid which affects exclusively the cardiac, skeletal and smooth muscle tissues of the body. Those cases in which amyloid infiltration is confined primarily to the heart constitute the final group.

Since the condition in the present case was not appreciated at the time of postmortem examination extensive investigation of the skeletal muscles of the head, extremities and trunk was not made, although the smooth muscle of the bladder and gastro-intestinal tract was examined both grossly and microscopically. The absence of clinical evidence of skeletal muscle disease, and the finding of amyloid only in the locations described, would seem to warrant the conclusion that this case is one of primary amyloidosis of the heart. The amount of amyloid present in the myocardium is, perhaps, less than has been described in previous cases and, so far as is known, a partial heart block was the only specific clinical observation that might have been caused by the disease. The great importance of the heart lesion as a cause of death is questionable, but it undoubtedly contributed to the final cardiac failure.

Summary

1. A case of primary amyloid disease of the heart is reported both descriptively and pictorially.

2. Mayer's method for staining amyloid in paraffin sections is described.

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DESCRIPTION OF PLATES

PLATE 83

FIG. 1. Right auricle. Note amyloid in the auricular endocardium, superior vena cava, valve of the coronary sinus, and the Chiari network. Involvement of the tricuspid valve is seen only near the margin of attachment. Iodine stain; about $\frac{3}{4}$ natural size.

FIG. 2. Right ventricle, conus arteriosis, pulmonary valve and pulmonary artery. Iodine stain; $\frac{3}{8}$ natural size.

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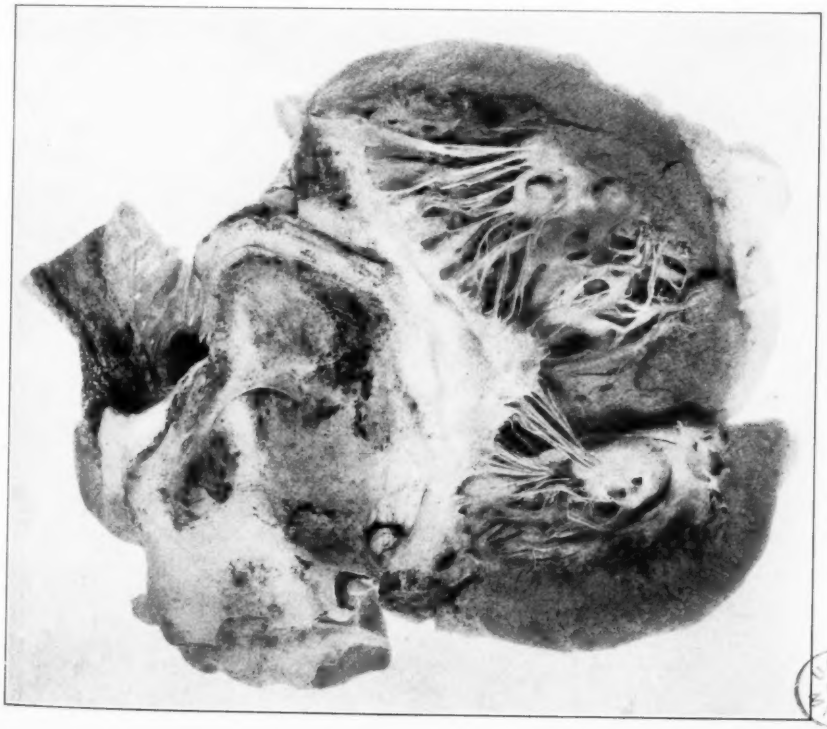
Primary Amyloid Disease of Heart

PLATE 84

FIG. 3. Left auricle, mitral valve and left ventricle. Iodine stain; $\frac{5}{8}$ natural size.

FIG. 4. Cross-section of left ventricle. Iodine stain. × 3.5.

4



3

Budd

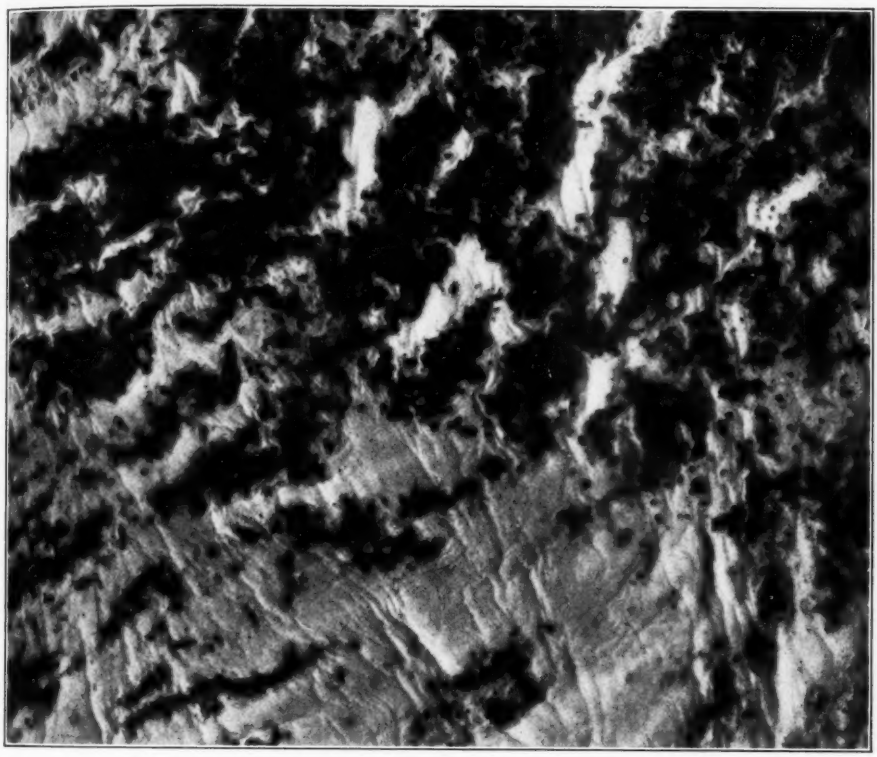
Primary Amyloid Disease of Heart

Plate 85

Fig. 5. Surface of endocardium of right auricle. Iodine stain. × 13.

Fig. 6. Cut surface of myocardium of right auricle. Iodine stain. × 18.

Fig. 7. Cross-section of pulmonary artery. Gentian violet stain. × 25.



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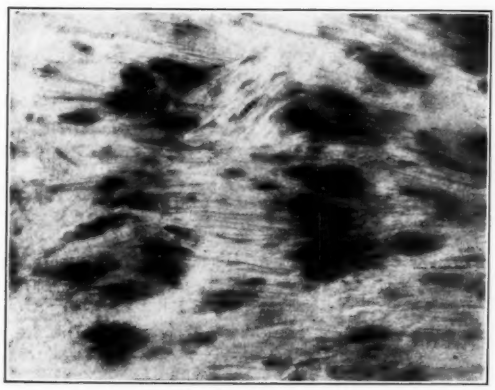

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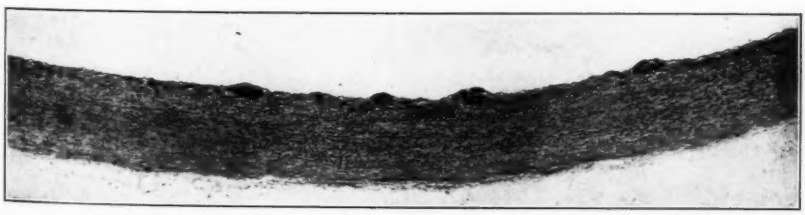

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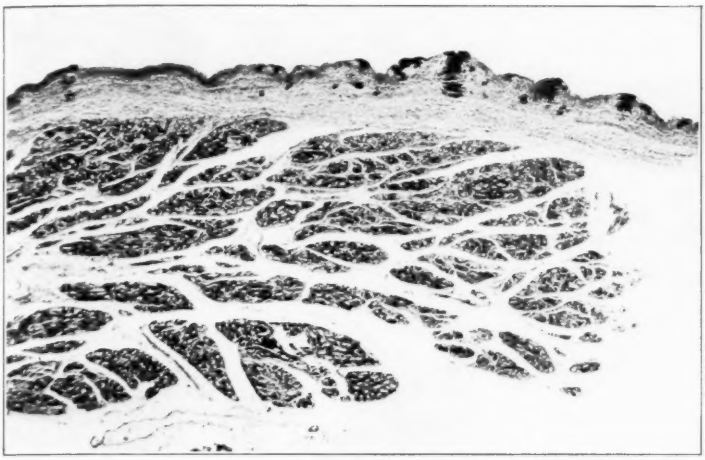
Budd Primary Amyloid Disease of Heart

PLATE 86

FIGS. 8 and 9. Cross-sections of right auricle. Gentian violet stain. × 25.

FIG. 10. Cross-section of right auricle. Gentian violet stain. × 125.



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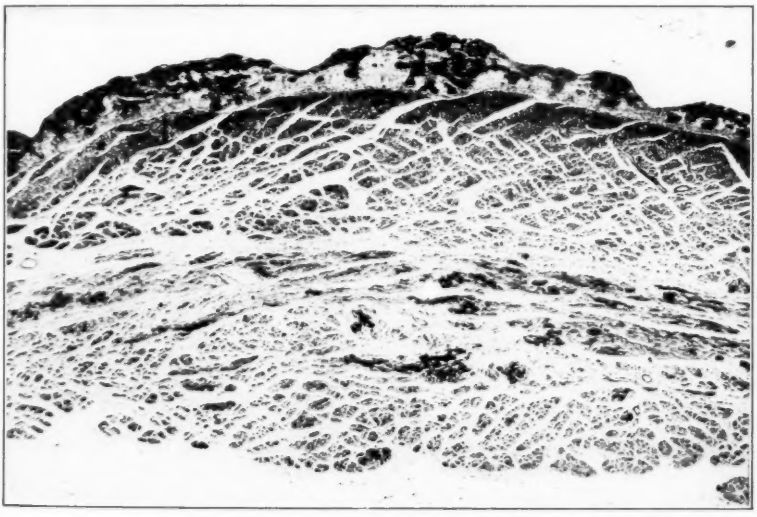

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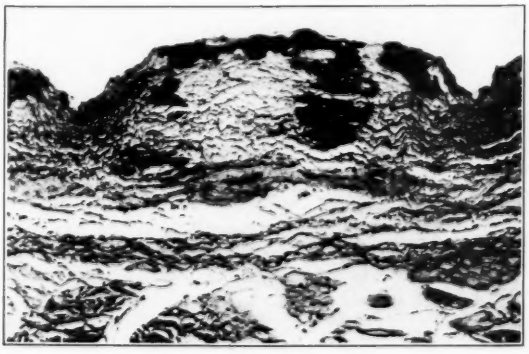

10

A CASE OF HODGKIN'S DISEASE IN A DOG *

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One hundred years have passed since Hodgkin [1] described clinically a disease of the lymphoid system that later acquired his name. During this period the literature has abounded with papers dealing with various aspects of this problem, perhaps most outstanding of which are the works of Wilks,[2] Sternberg,[3] Reed,[4] and Ziegler.[5] The variety of clinical manifestations, the typical as well as the atypical histological types, the time honored discussion of the relation of this disease to tuberculosis, the problem of etiology, and lastly the question of whether or not this disease should be considered as a type of malignant tumor are all problems with which both clinicians and pathologists are only too familiar, and will not be discussed in this very brief report. With the literature as extensive as it is, it is quite possible that some papers may have been overlooked, but after a careful search I have been unable to find a single report of Hodgkin's disease occurring in the dog.

We have recently found a case, quite by chance, in a dog brought into the school for experimental purposes. The object of reporting this case is not to call attention to what would appear to be rather a curiosity, but to point out that a disease of lymph nodes, simulating Hodgkin's lymphogranulomatosis in man, may occur spontaneously in dogs.

The dog was of the large mongrel type and showed on examination a firm nodular swelling approximately 7 cm. in diameter along the right side of the neck. This swelling could be grasped in the hand and moved slightly, but it seemed definitely adherent to the overlying skin, which at one point showed a recent abrasion. A complete autopsy was performed which, with the exception of this mass in the neck, may be considered negative. Sections were taken from several areas in this mass and stained with a variety of stains to bring out cellular and intercellular detail; in addition a futile

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search was made for tubercle bacilli, both by direct smear and also in fixed tissue preparations.

Grossly the swelling consisted of a massive enlargement of the cervical lymph nodes, in the midst of which the individual nodes could not be defined. They were bound not only together, but also to the surrounding fascia and muscles. The mass varied in color, texture and consistence; some areas were soft, gray and homogeneous, others were gray, tough and fibrous. Still other areas, slightly caseous, suggested areas of necrosis.

Histologically, the normal architecture of the lymph nodes is almost entirely replaced by a very cellular type of granulation tissue showing a complex hyperplasia of endothelial cells, reticular cells, fibroblasts, lymphocytes, plasma cells, eosinophiles and, most characteristic of all, many large cells well recognized as the Sternberg or Dorothy Reed giant cells. These cells are both single and multinucleated with abundant, homogeneous blue-staining cytoplasm and large, round, oval or irregular nuclei showing not infrequently symmetrical and asymmetrical mitoses. Some areas are composed almost entirely of lymphocytes and plasma cells, others of reticular and endothelial cells supported by a delicate reticulum, still other areas much less cellular are sclerosed and made up of coarse bands of collagen fibers, isolating here and there typical Sternberg giant cells. The vessels are large and thin-walled, and in some areas where collections of cells have accumulated beneath the endothelium of the intima the lumina are almost obliterated. The capsule of the lymph nodes is infiltrated and the same confusion of cell types is seen out in the surrounding connective tissue and muscles.

The location of the lesion and the complexity of the histological picture, coupled with the presence of the characteristic Sternberg giant cells permit, from the standpoint of microscopy, the diagnosis of Hodgkin's granuloma almost with certainty.

The restricted localization of the disease in this case may lead one to doubt this diagnosis or to accept it with some hesitancy. Originally Hodgkin's disease was looked upon as a fairly disseminated lesion of lymphoid tissue, and probably even today most cases fall into this category. However, many cases are now diagnosed both by clinicians and by pathologists in which only a single group of lymph nodes are involved, or perhaps only a single organ or system of organs.

In a condition such as Hodgkin's disease, in which the etiology is still obscure, the final diagnosis must depend on the histological examination. Furthermore, as Sternberg [6] has recently pointed out, in criticizing many of the so-called atypical types of Hodgkin's disease one must find the characteristic histological changes considered specific for the disease before one can establish a specific histological diagnosis.

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DESCRIPTION OF PLATE

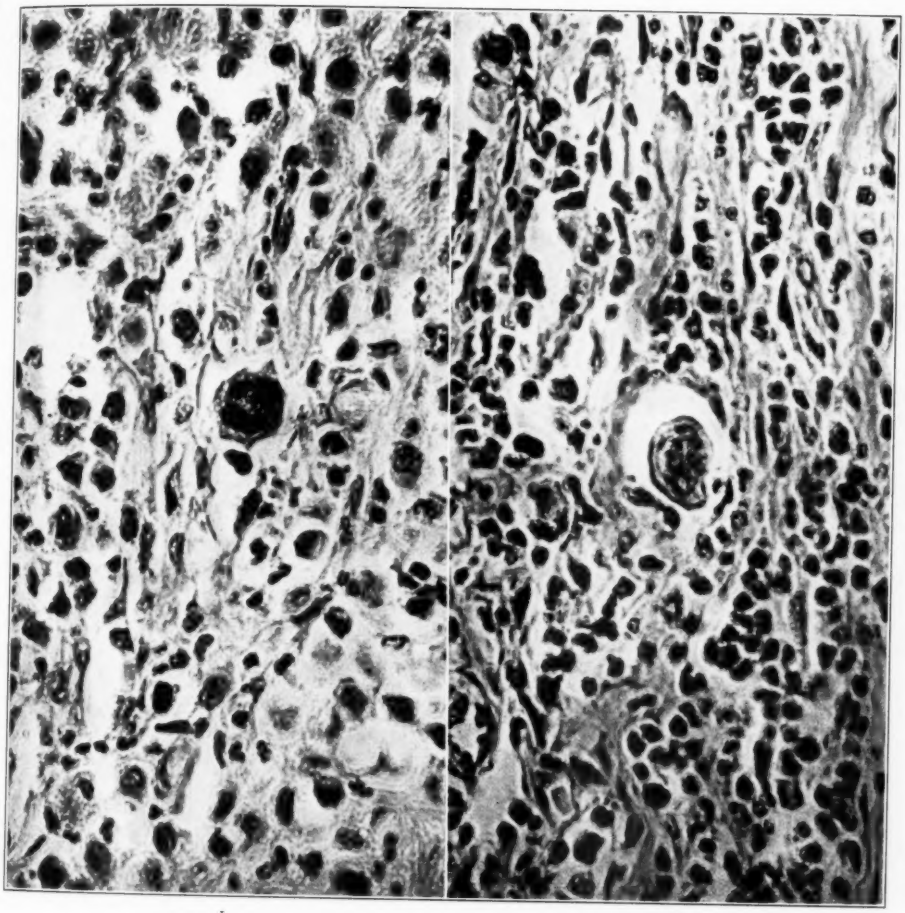
PLATE 87

FIG. 1. Lymph node. In the center of the field is a large Sternberg giant cell surrounded by lymphocytes, reticular cells and fibroblasts. The background is composed of a delicate reticulum infiltrated with polymorphonuclear leukocytes, including an occasional eosinophil.

FIG. 2. Lymph node. In the center of the field is a large Sternberg giant cell surrounded by lymphocytes, and an occasional large reticular cell. The stroma is abundant and is composed of interlacing bundles of collagen fibrils.

FIG. 3. Lymph node. Section shows a large Sternberg giant cell in the center, surrounded by lymphocytes, fibroblasts, a few polymorphonuclear leukocytes and an occasional swollen reticular cell. The connective tissue stroma is fairly abundant.

FIG. 4. Lymph node. Section shows four Sternberg giant cells, scattered lymphocytes, fibroblasts, an occasional polymorphonuclear cell, and a rather delicate reticulum stroma.



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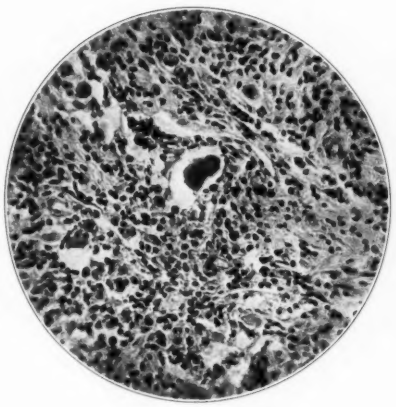

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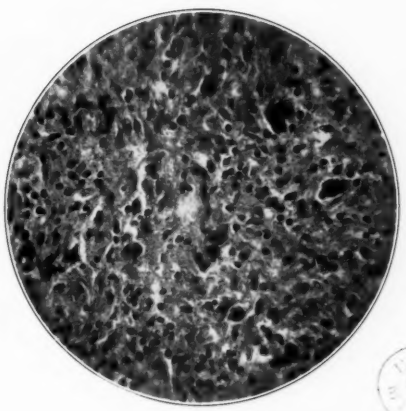

4

MacMahon

Hodgkin's Disease in a Dog

PRIMARY CARCINOMA OF THE DUODENUM *

VINCENT J. DARDINSKI, M.D.

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Primary carcinoma rarely attacks the small intestine and when it does it is usually found in the duodenum or close to the ileocecal valve. From 1915 to 1919 inclusive Lahey,[1] Jefferson,[2] and Judd[3] reported 24 cases of primary carcinoma in the small intestine distributed as follows: 11 cases were in the jejunum, 5 in the duodenum, 6 in the ileum and 2 showed multiple involvement. In 1925 Eusterman, Berkman and Swan[4] reported 39 cases of primary carcinoma of the small intestine, of which 15 were in the duodenum.

The frequency with which carcinoma of the duodenum is found can be judged by the fact that between the years 1882 to 1931 inclusive there have been reported in the literature 6882 cases of intestinal carcinoma. Of these 147 or 2.13 per cent were in the duodenum. This is roughly 0.01 to 0.1 per cent of all postmortem examinations. The figures are usually given as 0.01 to 0.03 per cent.[5] According to Brill,[6] only 2.5 per cent of intestinal carcinomas are in the small intestine, being equally divided between the duodenum and the ileum.

For purposes of description carcinoma of the duodenum is divided into three groups, according to their anatomical position: (1) parapyloric, (2) peri-ampullary, and (3) prejejunal. They may be more conveniently classified as carcinoma of the first, second or third portion of the duodenum, or according to their relation to the major duodenal papilla as suprapapillary, peripapillary, and infrapapillary. Of these by far the most common type is the peripapillary, the most infrequent the infrapapillary or prejejunal.

The suprapapillary type has practically the same clinical picture as carcinoma of the pyloric end of the stomach and since it is relatively uncommon in this position and because of its close relation to the pylorus it is probably suspected less commonly than carcinoma of the pylorus. It usually encircles the duodenum, producing complete or almost complete stenosis with dilatation of all the bowel proximal to it, including the pyloric ring.

* Received for publication August 26, 1933.

The peripapillary is the most frequent type of carcinoma of the duodenum. It usually arises in or about the papilla as an infiltration and tends to encircle and extend along the duodenum. If it occurs in the upper part of the second portion of the duodenum or just above the major duodenal papilla, and not involving it, practically the same clinical picture will be produced as with carcinoma in the suprapapillary portion, or pyloric carcinoma. If it involves the papilla the flow of bile will be interfered with and jaundice or other complications will develop. If it is immediately below the papilla stenosis will gradually occur and give rise to obstruction with bilious vomiting.

It must also be remembered that carcinoma just above the papilla may produce the same clinical symptoms as that occurring on or in the papilla. This is due to the close proximity of the common bile duct to the wall of the intestine in this region, as a result of which it may gradually be involved and occluded by fibrous stricture or by direct extension of the growth into the duct. However, carcinoma above the papilla may be differentiated from carcinoma of the papilla because of the gradual development of persistent jaundice, in contrast to the intermittent type of jaundice usually seen with carcinoma of the papilla.

The term "carcinoma of the ampulla of Vater" is sometimes erroneously applied to carcinoma of the duodenum involving the papilla. It should be understood that by the former term is meant carcinoma arising inside the papilla, while the other type is on the surface of the papilla and of the intestinal mucosa. When the carcinoma arises on the surface of the papilla the flow of bile is interfered with, although not as a rule completely blocked. A change that may frequently follow on this and rapidly prove fatal is suppurative inflammation of the bile ducts.

The infrapapillary type of carcinoma is the least frequent. It usually is in close proximity to the ligament of Treitz and produces symptoms similar to those of intermittent intestinal obstruction. It resembles pyloric obstruction, except for the presence of bile and pancreatic juice in the vomitus.

It is not the purpose of this paper to enter into a discussion concerning the type of tissue from which the newgrowth arises. Suffice it to say that ulcer, aberrant tissues or fetal rests may be responsible, but because of the extreme difficulty with which the pathologist is

confronted in trying to differentiate them it seems sufficient at present to call these newgrowths adenocarcinoma, until better methods are developed which will enable us to be more accurate in a differentiation.

The following case we believe is worthy of reporting because it represents a primary carcinoma of the duodenum of the periampullary type occurring in close proximity to the papilla but not involving it.

Report of Case

Clinical History: The patient, C. P., a male negro, 45 years of age, was admitted to the Georgetown University Hospital with a history of progressive painless jaundice of 3 weeks duration, accompanied by belching, heartburn, sour stomach and vomiting on one occasion. There had been progressive weakness, loss of weight (18 pounds), and diarrhea for 3 weeks, but no bloody or tarry stools had been present. Previous health up to time of onset of present symptoms was excellent.

Physical examination disclosed a marked icterus of the sclerae, and pronounced weakness. The abdomen was slightly distended and a small mass about 8 cm. in diameter was felt in the gall-bladder region.

The urine contained a large amount of bile, albumin and many casts. The blood contained 68 per cent hemoglobin, 3,330,000 red blood cells and 7400 white blood cells, of which 80 per cent were polymorphonuclear. The Wassermann reaction was negative. The van den Bergh test was direct, prompt; indirect 60 mg. On X-ray study a dilated stomach and duodenal cap were seen. Following barium injection there was 60 per cent retention, and vomiting became markedly aggravated.

The patient was operated upon a week after admission. A mass was found in the region of the head of the pancreas. A posterior gastrojejunostomy and cholescystotomy were performed for duodenal obstruction, believed to be due to a duodenal carcinoma. Death occurred 2 days later as the result of toxemia and bronchopneumonia.

Postmortem Examination

At autopsy the liver weighed 1890 gm. It was markedly jaundiced and very soft, making the normal markings indistinct. The gall-bladder was normal but contained a rubber tube drain sutured in the fundus. The common duct was markedly dilated and obstructed in its lower third (Fig. 1). Dissection of this obstructed portion of the duct disclosed a patent lumen with a few small nodules in the mucosa measuring about 2 mm. in diameter. This part of the common duct was firmly adherent to the adjacent portion of the duodenum and was surrounded by dense fibrous tissue. The duodenal wall at this point was firm and presented a hard ring

around the entire circumference. The bowel proximal to this ring was greatly dilated and distal to it was collapsed. On opening the duodenum a circular area of induration about 5 cm. in diameter was present 2 cm. above the major duodenal papilla. The induration extended through the wall of the bowel, producing a marked thickening and forming a ring, as mentioned above. The extraduodenal portion of the common bile duct was involved in this mural thickening and became obstructed by it. No evidence of metastasis or glandular involvement was found.

The head of the pancreas was normal and without evidence of involvement.

Other important findings at autopsy were a well developed bronchopneumonia and complete transposition of the colon.

The most important microscopic findings were a bronchopneumonia and a low grade adenocarcinoma in the area of induration in the duodenum. The entire wall of the duodenum in the region of induration was invaded by large, irregular, well developed glandular acini composed of tall columnar epithelium. The wall of the common duct was invaded by similar glandular structures. Considerable fibrous tissue was present, intermingled with the carcinomatous growth (Fig. 2).

Summary

Statistics showing the frequency of duodenal carcinoma are presented, together with salient factors that are necessary when one attempts to differentiate neoplasms of the suprapapillary, peripapillary, and infrapapillary areas of the small bowel.

A case report is also given in which a low grade adenocarcinoma of the duodenum was found 2 cm. above the major duodenal papilla. The clinical signs and symptoms simulated those of carcinoma of the head of the pancreas and carcinoma arising in the major duodenal papilla.

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DESCRIPTION OF PLATE

PLATE 88

FIG. 1. Photograph of the gross specimen dissected along the course of the common duct to determine the point of obstruction.

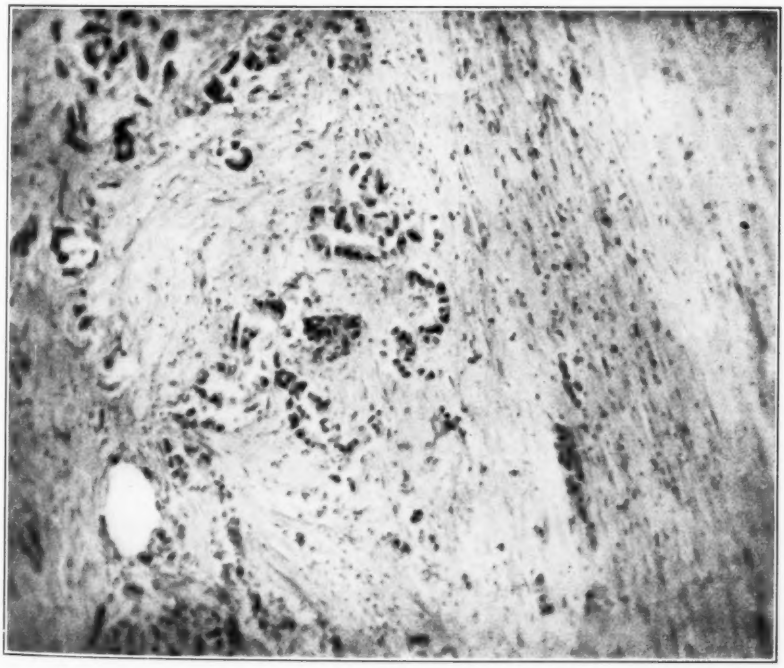
A, represents the dilated common duct.

B, shows the point of constriction of the duct at which obstruction occurred. The constriction was due to the carcinoma encircling the duct.

C, at this point can be seen the marked thickening of the duodenal wall produced by the carcinoma and fibrous tissue, as seen in Fig. 2.

FIG. 2. Photomicrograph of a section taken at point C in Fig. 1. In it can be seen differentiated acini of columnar epithelium lying in a loose fibrous stroma. Similar acini were found throughout the entire thickness of the bowel. It represents a low grade adenocarcinoma.

1



2

Dardinski

Primary Carcinoma of the Duodenum

ADDENDA TO A THEORY OF PIGMENTED MOLES

GEORGE F. LAIDLAW, M.D., AND MARGARET R. MURRAY, PH.D.

MIESCHER'S PAPER

After our paper on the theory of pigmented moles [1] had been sent to press Professor Masson, visiting this laboratory, called our attention to the confirmation of his observations by Miescher [2] in a recent volume of Jadassohn's great Handbuch. Miescher's paper is undoubtedly the best written and best illustrated discussion of pigmented moles that has appeared in recent years. Like Masson, Stout, Ewing and Foot, Miescher finds neural plexuses and groups of tactile corpuscles in many pigmented moles and he justly insists that in order to demonstrate these structures the moles must be properly fixed and stained. He announces a more detailed study of the neural structures in pigmented moles by Miescher and von Albertini, to appear in *Virchows Archiv*, 1933 (not yet published).

From Miescher's paper we select this significant paragraph: "In their structure and in the relation of the different tissue elements to one another, pigmented moles differ so absolutely from all known microscopic pictures that it is difficult to understand their nature and to comprehend their significance." (See page 1015.)

It is true that if we restrict our studies, as Miescher has done and as every other histologist has done, to mammalian skin, the structure of a pigmented mole is absolutely incomprehensible. But if we look at sections of amphibian or reptilian skin we shall see each puzzling feature of the human pigmented mole fall naturally into place as part of a normally functioning amphibian or reptilian tactile terminal.

Referring to our own paper, compare the impression produced by our Figures 1 to 5, the neural structures in human pigmented moles, with Figures 7 and 8, sections through normal reptilian tactile spots. In Figures 1 to 5 the groups of innervated tactile cells in the derma, the intermingling columns of schwann cells, the threadlike terminal nerves running in all directions, the irregular accumulation of pigment, — all this in human skin is, as Miescher says, a meaningless

jumble. In the reptilian skin of Figures 7 and 8 the intermingling of exactly these same structures constitutes a well ordered and highly efficient tactile terminal.

A Chronological Parallel

In connecting the ontogeny of the pigmented mole with that of the hair follicle we explain the hitherto puzzling phenomenon of hereditary and "congenital" pigmented moles appearing at intervals during the entire life of the individual. The periodical eruption of pigmented moles parallels the periodic outbursts of new hair follicles. It is well known that pigmented moles appear most abundantly at birth or shortly thereafter, next at puberty, and then in diminishing numbers scattered through later years. This is precisely the sequence of activity in the extension of hair follicles over new areas. Obviously the factors, probably of hormonal nature, which induce the formation of new groups of hair follicles (mammalian tactile organs) operate at the same time on those aberrant groups of cells that already have been determined for the formation of the reptilian type of tactile organ — the pigmented mole.

In accord with this observation are data from experimental embryology which show that the time order of the events of segregation, *i.e.*, determination of ultimate potencies of cell groups, is quite independent of the actual organogenesis, which often occurs much later and whose time of incidence is dependent upon numerous factors operating in the developing and functioning organism.

In addition to the chronological parallel between hair follicle and mole there is another, that of pigment formation. Of all derivatives of the epidermal ectoderm, normal or pathological, the hair matrix and the pigmented mole alone exhibit regularly and prominently the melanogenic properties of the surface epithelium.

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